U.PORTO

FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2017/2018

Ana Teresa Pinto e Silva

Rapid flow cytometry test for identification of different carbapenemases in Enterobacteriaceae

Teste rápido para identificação das diferentes carbapenemases em Enterobacteriaceae por citometria de fluxo

março, 2018

Ana Teresa Pinto e Silva

Rapid flow cytometry test for identification of different carbapenemases in Enterobacteriaceae

Teste rápido para identificação das diferentes carbapenemases em Enterobacteriaceae por citometria de fluxo

Mestrado Integrado em Medicina

Área: Microbiologia

Tipologia:Dissertação

Trabalho efetuado sob a Orientação de:

Professora Doutora Cidália Irene Azevedo Pina Vaz

Trabalho organizado de acordo com as normas da revista:

Antimicrobial Agents and Chemotherapy

março, 2018

U.PORTO
FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, Ana Teresa Pinto e Silva, abaixo assinado, nº mecanográfico 200000044, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 20/03/2018

Assinatura conforme cartão de identificação:

Ana Teresa Pinto e Silva

U.PORTO
FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

Ana Teresa Pinto e Silva

NÚMERO DE ESTUDANTE	E-MAIL
200000044	atsilva@med.up.pt

DESIGNAÇÃO DA ÁREA DO PROJECTO

Microbiologia

TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

Rapid flow cytometry test for identification of different carbapenemases in Enterobacteriaceae

ORIENTADOR

Professora Doutora Cidália Irene Azevedo Pina Vaz

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE. -	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☒

Faculdade de Medicina da Universidade do Porto, 20 / 03 / 2018

Assinatura conforme cartão de identificação: Ana Teresa Pinto e Silva

À minha Mãe

Rapid flow cytometry test for identification of different carbapenemases in Enterobacteriaceae

Ana Pinto Silva[a,b,#], Isabel Faria-Ramos[a,b], Elisabete Ricardo[a,b], Isabel Miranda[a,b], Maria José Espinar[a,b,c], Sofia Costa-de-Oliveira[a,b], Rafael Cantón[d], Acácio Gonçalves Rodrigues[a,b] and Cidália Pina-Vaz[a,b,c]

[a]Microbiology Division, Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal

[b]Center for Research in Health Technologies and Information Systems (CINTESIS), Porto, Portugal

[c]Microbiology Department, Centro Hospitalar São João, Porto, Portugal

[d]Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain

Running Title: Carbapenemases identification by flow cytometry

[#]Address correspondence to Ana Pinto Silva, atsilva@med.up.pt.

Abstract

Enterobacteriaceae producing carbapenemases, such as KPC or metallo-β-lactamases, have emerged on several continents. Phenotypic tests are urgently needed for their rapid and accurate detection. A novel and fast flow cytometry test was developed in order to identify carbapenemase production by Enterobacteriaceae and to discriminate between the different types of carbapenemases belonging to Ambler molecular classes A, B, and D. It is based upon the comparison of meropenem antimicrobial activity alone and coupled with different carbapemase inhibitors (aminophenylboronic acid and EDTA), assessed by flow cytometry. Decreased susceptibility to carbapenems caused by either ESBL or AmpC enzymes combined with decreased permeability was also detected using temocillin and cloxacillin, overcoming the false positive results obtained with phenotypic carbapenemase detection tests. The test was applied directly to 60 bacterial colonies, half of them carbapenemases producers (KPC, VIM, IMP, NDM, IMI, GIM, OXA-48-like, and KPC plus VIM). The test yielded sensitivity and specificity of 100%, distinguished accurately between several different mechanisms mediating reduced susceptibility to carbapenems in Enterobacteriaceae. It represents a convenient and reliable phenotypical approach for detection and characterization of different carbapenemases with a time-to-results of 2 hours *versus* 24 hours of classic methods.

Introduction

In the last decade, the prevalence of resistance to β-lactam antibiotics mediated by β-lactamases has increased at an alarming rate, especially among Enterobacteriaceae (1); firstly, because of the spread of extended spectrum β-lactamases (ESBL) and more recently due to carbapenemases, which are enzymes able to hydrolyze carbapenems. These enzymes have also spread rapidly throughout the world (2), being an alarming problem in some countries, especially in Greece and India (3). In 2015, 13/38 countries reported inter-regional spread of or an endemic situation for carbapenemase-producing Enterobacteriaceae (CPE), compared with 6/38 in 2013. Only three countries replied that they had not identified one single case of CPE (4). Carbapenemases include Ambler class A (such as *Klebsiella pneumoniae* carbapenemase, KPC), Ambler class B (metallo-β-lactamases, MBL, such as VIM-type, IMP-type, NDM-type) and Ambler class D (OXA-48-types, such as OXA-181) enzymes (Table 1). Invasive infections with CPE are associated with high morbidity and mortality (5, 6). Carbapenemases may confer resistance to virtually all β-lactams, and are readily transferable. Additional, CPE frequently possess resistance mechanisms to a wide-range of antimicrobial agents, since such enzymes hydrolyze penicillins, in most cases cephalosporins, and to various degrees carbapenems and monobactams (the latter are not hydrolyzed by metallo-β-lactamases). Accurate detection of CPE is relevant at individual patient not only due to the impact on antibiotic therapy but also for infection control purposes, especially in outbreak settings. The European Comittee of Antimicrobial Susceptibility Testing (EUCAST) recently developed guidelines for carbapenemase detection in Enterobaceriaceae isolates (7, 8). These recommendations must be adopted whenever decreased susceptibility to imipenem, meropenem and/or ertapenem is detected, meaning Minimal Inhibitory Concentration (MIC) values >1, >0.125 and >0.125 mg/L,

respectively; values below the clinical breakpoints for susceptible phenotype. In the most recently recommendations (8), imipenem was not considered and meropenem is the carbapenem that offers the best compromise between sensitivity and specificity in terms of detecting carbapenemase-producers (9). However, it usually requires 48 hours after sample collection to determine the MIC value and an additional 18-24 hours to confirm the presence of carbapenemase. Combination disk test is the unique most convenient phenotypic method to discriminate different carbapenemases (10-12). In brief, disks or tablets containing meropenem without and with various inhibitors such as aminophenyl boronic acid (APBA) as a class A inhibitor and ethylenediaminetetraacetic acid (EDTA) as a class B inhibitor are used. No inhibitor to OXA-48-type enzymes was included in the phenotypic panels. However, these enzymes confer high-level resistance to temocillin (12). Decreased susceptibility of Enterobacteriaceae to carbapenems may be also caused by either the production extended spectrum β-lactamases (ESBL) or AmpC β-lactamases coupled with decreased permeability due to alteration or down-regulation of porins (13). Cloxacillin, which inhibits AmpC β-lactamases, should be added to the test in order to differentiate between AmpC hyperproduction plus porin loss and carbapenemase-production. Figure 1 represents an algorithm for interpretation of results with β-lactamases inhibitors recommended by EUCAST. The main disadvantage of such procedure is that it requires a long incubation period (18-24 hours), since it is based on growth in presence of the drugs. The same drawback is appointed to Etest KPC and MBL strips, which associate a carbapenem with boronic acid or EDTA, respectively (14). A possible alternative is the carbapenem inactivation method (i.e. CIM-test), which has had variable performance in different studies and requires at least 18 hours to the result (15). The market available chromogenic culture media although easy to perform are also growth dependent (16).

More recently, different biochemical test such as Carba NP was described to detect carbapenemase production (17, 18); it seems to be rapid (<2h), sensitive and specific although cumbersome and expensive and a discrimination among carbapenemase-types is not possible. MALDI-TOF MS, recently introduced in the laboratory routine, seems promising in order to detect antibiotic modifications due to degradation by enzymes, including carbapenemases (19, 20). A new immunochromatographic lateral flow assay is available for direct identification OXA-48-like enzymes and KPC, but its robustness has not been evaluated so far (21). Molecular identification of carbapenemase genes (22, 23) is another possibility but, besides the several genes that must be searched and high associated costs, it can result in false negatives when mutations or new genes emerge.

Flow cytometry (FC) represents an accurate and fast approach for the analysis of cell architecture and its functional parameters, with considerable advantages over conventional methods (24). Similar to ESBL detection by FC (25), a novel protocol for carbapenemase detection, with time-to-results within 2 hours, was hereby developed and evaluated, using a comprehensive set of phenotypically and molecularly well-characterized strains.

Results

An increase (of at least twice) in fluorescence intensity, meaning cell lesion, in the presence of inhibitors, when compared with the intensity in the presence of meropenem alone, was observed with APBA for all KPC, corresponding to class A carbapenemases. All VIM, IMP, NDM, IMI and GIM showed an increase in intensity of fluorescence when cells were treated with meropenem associated with EDTA, corresponding to class B carbapenemases. Strains with both KPC and MBL enzymes

had an increase in intensity of fluorescence when cells were treated with meropenem associated only with APBA plus EDTA. For class D carbapenemases no increase was observed however, they were resistant to temocillin; the fluorescence intensity of cells exposed to temocillin was similar to non-exposed cells. For AmpC associated with porin loss, the increase in cells intensity of fluorescence was observed with APBA but also with cloxacillin. In case of ESBL associated with porin loss no increase in intensity of fluorescence was observed; such strains were susceptible to temocillin, increasing fluorescence intensity of cells exposed to this drug. In Figure 2 a graphic showing the median values of intensity of fluorescence for each type of carbapenemases and for a group of non-producers is represented. A typical example of the different types concerning flow cytometry data are shown in figure 2. This FC kit combines excellent sensitivity (100%), specificity (100%) and short time to result.

Discussion

Carbapenemases are considered to be of high epidemiological importance, particularly when they confer decreased susceptibility to carbapenems. It is high recommended the application in clinical routine of phenotypic methods for detection of carbapenemases. The main categories of methods available have disadvantages encouraging the development of novel rapid methods. The rapid phenotypic tests been so far developed have limitations. The colorimetric tests (CarbaNP test, Blue-Carba test or β CARBA test™) only detect the carbapenemase production not allowing the distinction among the different classes and lack sensitivity for Enterobacteriaceae producing OXA-48 (17, 26, 27). Similar disadvantages are also appointed to MALDI-TOF technology for detection of carbapenem hydrolysis (19, 20). A new immunochromatographic lateral flow assay although promising its robustness has not

been evaluated so far (21, 28). FC already demonstrated to be an excellent tool in microbiology (24), overcoming the methods in use in routine clinical laboratories depending on growth; it gives a quicker and much more informative antimicrobial phenotype. The present work described the performance of a new method based upon FC that enables the fast determination of carbapenemases. The test exhibited 100% of sensitivity and specificity directly from bacterial colonies. It has the potential to be applied directly from blood cultures, saving extra 24 hours for bacterial isolation after sample collection. Indeed, a novel FASTinov® kit for antimicrobial susceptibility testing (AST) of Gram negative bacilli directly on positive blood culture was already tested and exhibited an overall agreement of 98% with the reference microdilution method (29). Moreover, the impact of the use of a rapid AST in clinical routine was evaluated resulting in a reduced in hospital length of stay, lower consumption of antibiotics and in ultimately case improved infection control. Recently, new data was obtained with the use of this methodology directly from blood culture. The kit was able to distinct the different types of carbapenemases (30).

The method although currently present as a semi-automated method, since it has dedicated software, it could be fully automatized. The estimated costs for this new method are rather low, around two and half euros per microorganism and the FC equipment is frequently found in most hematology/immunology laboratories. The early detection of carbapenemases could have a real impact on outbreaks as well as in antibiotic therapy (31, 32). The improvement in detection and surveillance of CPE might contribute for their epidemiological control.

Material and Methods

Bacterial strains

A panel of 30 carbapenemase-producing Enterobacteriaceae strains was used in this study: KPC (8), VIM (7), IMP (1), NDM (4), IMI (1), GIM (1), OXA-48-like (6), and KPC plus VIM (2). As non-carbapenemase producers, 30 Enterobacteriaceae strains, including several strains that give false positive results on phenotypic tests (9), like ESBL or AmpC producers coupled to impermeability, were included.

Inoculum preparation

Bacterial suspensions, of the 60 strains described above, were prepared in Brain-Heart medium (Merck) with approximately 10^6 cells/mL at initial log phase, ($O.D._{600nm} \geq 0.2$, which takes approximately 1.5 hours).

Flow cytometric kit inoculation

A novel flow cytometric kit for carbapenemase detection (provided by FASTinov Ltd, Porto, Portugal) was used. Briefly, bacterial cells obtained directly from colonies were incubated with meropenem (2 and 8 µg/mL) with and without the inhibitors APBA (2.5 and 5 mM), EDTA (6.25 and 12.5 mM), APBA plus EDTA (2.5 plus 6.25 and 5 plus 12.5 mM) and cloxacillin (250 and 500 µg/mL). Temocillin (Eumedica S.A., Brussels, Belgium) alone, at 32 and 64 µg/mL was also included. The kit fluorochrome dye was disposed in all tubes. Cells were incubated for 1 hour at 37ºC and analyzed in a flow cytometer apparatus.

Flow cytometry analysis

Bacteria cells after exposure to antimicrobial treatments were analysed at a FACSCalibur flow cytometer (Becton Dickinson). The intensity of fluorescence of cells treated with meropenem alone was compared with those exposed to meropenem plus inhibitors.

Acknowledgments

We would like to thanks FASTinov S.A. (Porto, Portugal) for providing the FC kits and to Helena Ramos, MD, (Centro Hospitalar do Norte, Porto, Portugal) for providing strains. We are grateful to Isabel Santos for excellent technical assistance.

205 **References**

206 1. **Castanheira M, Mendes RE, Woosley LN, Jones RN.** 2011. Trends in
207 carbapenemase-producing Escherichia coli and Klebsiella spp. from Europe and
208 the Americas: report from the SENTRY antimicrobial surveillance programme
209 (2007-09). J Antimicrob Chemother **66:**1409-1411.

210 2. **Nordmann P, Cuzon G, Naas T.** 2009. The real threat of Klebsiella
211 pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis **9:**228-236.

212 3. **Dautzenberg MJ, Wekesa AN, Gniadkowski M, Antoniadou A,**
213 **Giamarellou H, Petrikkos GL, Skiada A, Brun-Buisson C, Bonten MJ,**
214 **Derde LP.** 2015. The Association Between Colonization With Carbapenemase-
215 Producing Enterobacteriaceae and Overall ICU Mortality: An Observational
216 Cohort Study. Crit Care Med **43:**1170-1177.

217 4. **Albiger B GC, Struelens MJ, Grundmann H, Monnet DL.** 2015.
218 Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national
219 experts from 38 countries, May 2015. Euro Surveill **20(45)**.

220 5. **Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, Quale J.** 2005.
221 Rapid spread of carbapenem-resistant Klebsiella pneumoniae in New York City:
222 a new threat to our antibiotic armamentarium. Arch Intern Med **165:**1430-1435.

223 6. **Souli M, Galani I, Antoniadou A, Papadomichelakis E, Poulakou G,**
224 **Panagea T, Vourli S, Zerva L, Armaganidis A, Kanellakopoulou K,**
225 **Giamarellou H.** 2010. An outbreak of infection due to beta-Lactamase
226 Klebsiella pneumoniae Carbapenemase 2-producing K. pneumoniae in a Greek
227 University Hospital: molecular characterization, epidemiology, and outcomes.
228 Clin Infect Dis **50:**364-373.

7. **European Committee on Antimicrobial Susceptibility Testing.** 2013. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. version 1.0.

8. **European Committee on Antimicrobial Susceptibility Testing.** 2017. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. version 2.0.

9. **Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V.** 2012. Identification and screening of carbapenemase-producing Enterobacteriaceae. Clin Microbiol Infect **18:**432-438.

10. **Giske CG, Gezelius L, Samuelsen O, Warner M, Sundsfjord A, Woodford N.** 2011. A sensitive and specific phenotypic assay for detection of metallo-beta-lactamases and KPC in Klebsiella pneumoniae with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. Clin Microbiol Infect **17:**552-556.

11. **Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD.** 2012. Laboratory detection of Enterobacteriaceae that produce carbapenemases. J Clin Microbiol **50:**3877-3880.

12. **van Dijk K, Voets GM, Scharringa J, Voskuil S, Fluit AC, Rottier WC, Leverstein-Van Hall MA, Cohen Stuart JW.** 2014. A disc diffusion assay for detection of class A, B and OXA-48 carbapenemases in Enterobacteriaceae using phenyl boronic acid, dipicolinic acid and temocillin. Clin Microbiol Infect **20:**345-349.

13. **Doumith M, Ellington MJ, Livermore DM, Woodford N.** 2009. Molecular mechanisms disrupting porin expression in ertapenem-resistant Klebsiella and

253 Enterobacter spp. clinical isolates from the UK. J Antimicrob Chemother
254 **63:**659-667.
255 14. **Girlich D, Halimi D, Zambardi G, Nordmann P.** 2013. Evaluation of Etest(R)
256 strips for detection of KPC and metallo-carbapenemases in Enterobacteriaceae.
257 Diagn Microbiol Infect Dis **77:**200-201.
258 15. **Yamada K, Kashiwa M, Arai K, Nagano N, Saito R.** 2016. Comparison of the
259 Modified-Hodge test, Carba NP test, and carbapenem inactivation method as
260 screening methods for carbapenemase-producing Enterobacteriaceae. J
261 Microbiol Methods **128:**48-51.
262 16. **Simner PJ, Gilmour MW, DeGagne P, Nichol K, Karlowsky JA.** 2015.
263 Evaluation of five chromogenic agar media and the Rosco Rapid Carb screen kit
264 for detection and confirmation of carbapenemase production in Gram-negative
265 bacilli. J Clin Microbiol **53:**105-112.
266 17. **Dortet L, Poirel L, Nordmann P.** 2012. Rapid identification of carbapenemase
267 types in Enterobacteriaceae and Pseudomonas spp. by using a biochemical test.
268 Antimicrob Agents Chemother **56:**6437-6440.
269 18. **Noel A, Huang TD, Berhin C, Hoebeke M, Bouchahrouf W, Yunus S,**
270 **Bogaerts P, Glupczynski Y.** 2017. Comparative Evaluation of Four Phenotypic
271 Tests for Detection of Carbapenemase-Producing Gram-Negative Bacteria. J
272 Clin Microbiol **55:**510-518.
273 19. **Lasserre C, De Saint Martin L, Cuzon G, Bogaerts P, Lamar E,**
274 **Glupczynski Y, Naas T, Tande D.** 2015. Efficient detection of carbapenemase
275 activity in Enterobacteriaceae by mass-spectrometry (MALDI-TOF, Bruker) in
276 less than 30 minutes. J Clin Microbiol.

20. **Hrabak J, Studentova V, Walkova R, Zemlickova H, Jakubu V, Chudackova E, Gniadkowski M, Pfeifer Y, Perry JD, Wilkinson K, Bergerova T.** 2012. Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 carbapenemases by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol **50:**2441-2443.

21. **Pasteran F, Denorme L, Ote I, Gomez S, De Belder D, Glupczynski Y, Bogaerts P, Ghiglione B, Power P, Mertens P, Corso A.** 2016. Rapid Identification of OXA-48 and OXA-163 Subfamilies in Carbapenem-Resistant Gram-Negative Bacilli with a Novel Immunochromatographic Lateral Flow Assay. J Clin Microbiol **54:**2832-2836.

22. **Findlay J, Hopkins KL, Meunier D, Woodford N.** 2015. Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. J Antimicrob Chemother **70:**1338-1342.

23. **Solanki R, Vanjari L, Subramanian S, B A, E N, Lakshmi V.** 2014. Comparative Evaluation of Multiplex PCR and Routine Laboratory Phenotypic Methods for Detection of Carbapenemases among Gram Negative Bacilli. J Clin Diagn Res **8:**DC23-26.

24. **C Pina-Vaz, S Costa-de-Oliveira, A Silva-Dias, AP Silva, R Teixeira-Santos and AG Rodrigues.** 2017. Flow Cytometry in Microbiology: The Reason and the Need, p 153-170. *In* Cossarizza JPRaA (ed), Single Cell Analysis: Contemporary Research and Clinical Applications.

25. **Faria-Ramos I, Espinar MJ, Rocha R, Santos-Antunes J, Rodrigues AG, Canton R, Pina-Vaz C.** 2013. A novel flow cytometric assay for rapid detection of extended-spectrum beta-lactamases. Clin Microbiol Infect **19:**E8-E15.

26. **Tijet N, Boyd D, Patel SN, Mulvey MR, Melano RG.** 2013. Evaluation of the Carba NP test for rapid detection of carbapenemase-producing Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrob Agents Chemother **57:**4578-4580.

27. **Compain F, Gallah S, Eckert C, Arlet G, Ramahefasolo A, Decre D, Lavollay M, Podglajen I.** 2016. Assessment of Carbapenem Resistance in Enterobacteriaceae with the Rapid and Easy-to-Use Chromogenic beta Carba Test. J Clin Microbiol **54:**3065-3068.

28. **Glupczynski Y, Evrard S, Ote I, Mertens P, Huang TD, Leclipteux T, Bogaerts P.** 2016. Evaluation of two new commercial immunochromatographic assays for the rapid detection of OXA-48 and KPC carbapenemases from cultured bacteria. J Antimicrob Chemother **71:**1217-1222.

29. **Costa-de-Oliveira S, Teixeira-Santos R, Silva AP, Pinho E, Mergulhao P, Silva-Dias A, Marques N, Martins-Oliveira I, Rodrigues AG, Paiva JA, Canton R, Pina-Vaz C.** 2017. Potential Impact of Flow Cytometry Antimicrobial Susceptibility Testing on the Clinical Management of Gram-Negative Bacteremia Using the FASTinov((R)) Kit. Front Microbiol **8:**2455.

30. **Costa-de-Oliveira S, Teixeira-Santos R, Silva AP, Silva-Dias A, Martins-Oliveira I, Rodrigues AG, Pina-Vaz C.** Evaluation of flow cytometric antimicrobial susceptibility test from FASTinov for detecting enzymatic resistance to beta-lactamic drugs on Enterobacteriaceae, abstract ID 7671 28th ECCMID

31. **Pina-Vaz C, Silva AP, Faria-Ramos I, Teixeira-Santos R, Moura D, Vieira TF, Sousa SF, Costa-de-Oliveira S, Canton R, Rodrigues AG.** 2016. A Flow

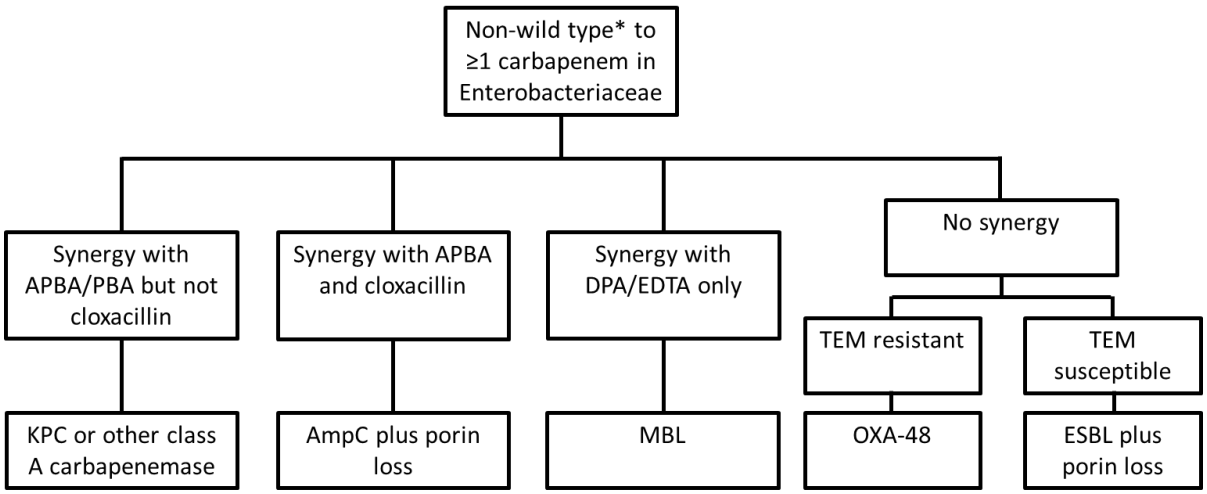
Cytometric and Computational Approaches to Carbapenems Affinity to the Different Types of Carbapenemases. Front Microbiol **7:**1259.

32. **Crandon JL, Nicolau DP.** 2013. Human simulated studies of aztreonam and aztreonam-avibactam to evaluate activity against challenging gram-negative organisms, including metallo-beta-lactamase producers. Antimicrob Agents Chemother **57:**3299-3306.

TABLE 1. Classification of carbapenemases according to Ambler molecular classes.

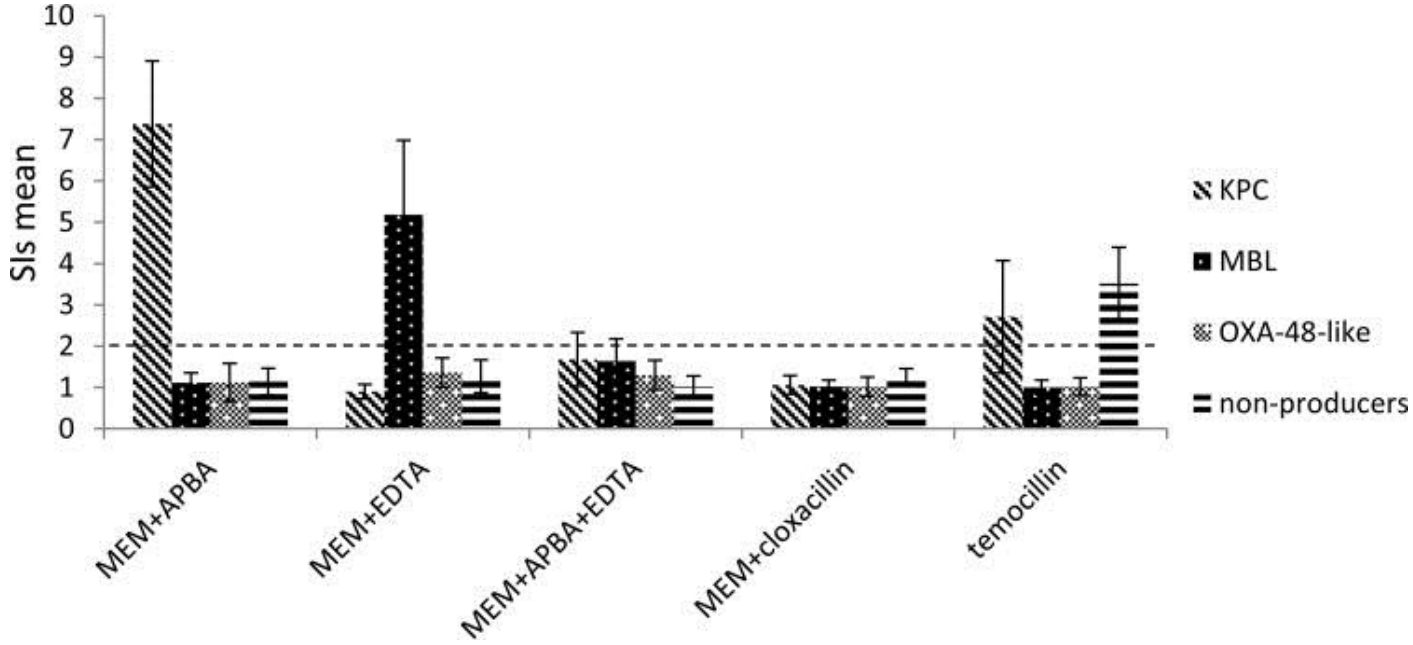
Molecular class	Enzyme
A	KPC, NMC, IMI, SME, GES
B	IMP, VIM, GIM, SPM, NDM
D	OXA

Figure 1. Algorithm for interpretation of phenotypic tests for carbapenemase detection with β-lactamases inhibitors. APBA, aminophenylboronic acid; PBA, phenyl boronic acid; EDTA, ethylenediaminetetraacetic acid; DPA, dipicolinic acid; MBL, metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase. *MIC above the epidemiological cut-off values defined by EUCAST.



395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412

Figure 2. Graphic representing the mean of stain indexs of each group of strains: KPC carbapenemases, MBL carbapenemases, OXA-48-like carbapenemases and non-producers, being the stain index the ratio between the intensity of fluorescence at FL1 of cells treated with the meropenem plus the inhibitor(s) and cells treated only with meropenem. Concerning temocillin the stain index is the ratio between cells treated with temocillin and cells no treated. For each strain the combination both drugs at lower concentration each was evident an increase in intensity of fluorescence was choose to performed the calculation.



435

436

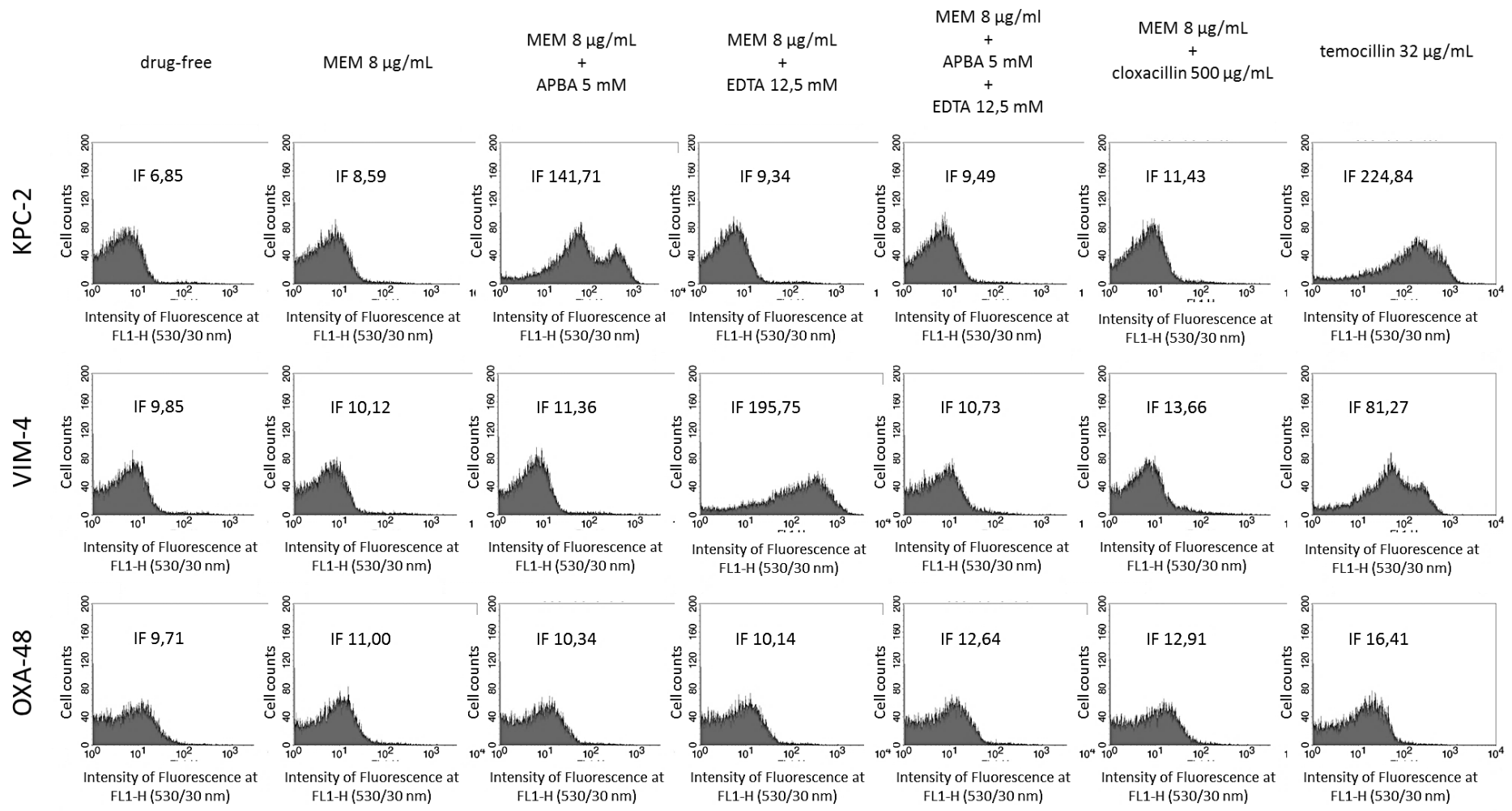
437

438

439

440

Figure 3. Representative histograms obtained by flow cytometry for different type of carbapenemases. For each strain (*Klebsiella pneumoniae* KPC-2, *Enterobacter cloacae* VIM-4 and *K. pneumoniae* OXA-48) it is represented the cells non-exposed to drugs, cells exposed to meropenem 8 µg/mL and with the inhibitors APBA 5 mM, EDTA 12,5 mM, APBA 5 mM plus EDTA 12,5 mM or cloxacillin 500 µg/mL and cells exposed to temocillin 32 µg/mL. IF, intensity of fluorescence.


drug-free
MEM 8 µg/mL
MEM 8 µg/mL + APBA 5 mM
MEM 8 µg/mL + EDTA 12,5 mM
MEM 8 µg/ml + APBA 5 mM + EDTA 12,5 mM
MEM 8 µg/mL + cloxacillin 500 µg/mL
temocillin 32 µg/mL
KPC-2
VIM-4
OXA-48
IF 6,85
IF 8,59
IF 141,71
IF 9,34
IF 9,49
IF 11,43
IF 224,84
IF 9,85
IF 10,12
IF 11,36
IF 195,75
IF 10,73
IF 13,66
IF 81,27
IF 9,71
IF 11,00
IF 10,34
IF 10,14
IF 12,64
IF 12,91
IF 16,41
Cell counts
Intensity of Fluorescence at FL1-H (530/30 nm)

Agradecimentos

Os meus sinceros agradecimentos,

À Professora Doutora Cidália Pina Vaz, orientadora do trabalho, pela partilha da sua sabedoria, pelas constantes palavras de incentivo e carinho e pelo seu apoio em momentos marcantes do meu percurso académico e pessoal.

Ao Professor Doutor Acácio Rodrigues, pelo seu contributo neste trabalho, pela amizade e pela confiança que em mim deposita.

Ao Grupo de Investigação da Microbiologia, pela colaboração neste trabalho, pelo apoio, companheirismo e pela partilha de bons momentos.

À Minha Família, em especial aos meus meninos, Miguel e Gabriel, pelo amor que nos une.

À minha doce Mãe e a Mãe de todos nós, Maria, que me amparam e ajudam a trilhar o meu caminho.

Normas da Revista

Antimicrobial Agents and Chemotherapy

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

January 2018, Instructions to Authors, pages 1–26

INSTRUCTIONS TO AUTHORS

SCOPE

Antimicrobial Agents and Chemotherapy ® (AAC) is an interdisciplinary journal devoted to the dissemination of knowledge relating to all aspects of antimicrobial and antiparasitic agents and chemotherapy. Within the circumscriptions set forth below, any report involving studies of or with antimicrobial, antiviral (including antiretroviral), antifungal, or antiparasitic agents as these relate to human disease is within the purview of AAC. Studies involving animal models, pharmacological characterization, and clinical trials are appropriate for consideration. Studies addressing species that are not pathogens for humans are out of scope unless the analysis has direct relevance for the treatment of human disease.

ASM publishes a number of different journals covering various aspects of the field of microbiology. Each journal has a prescribed scope that must be considered in determining the most appropriate journal for each manuscript. The following guidelines may be of assistance.

(i) Papers which describe the use of antimicrobial agents as tools for elucidating the basic biological processes of bacteria are considered more appropriate for the *Journal of Bacteriology*®.

(ii) Manuscripts that (a) describe the use of antimicrobial or antiparasitic agents as tools in the isolation, identification, or epidemiology of microorganisms associated with disease; (b) are concerned with quality control procedures for diffusion, elution, or dilution tests for determining susceptibilities to antimicrobial agents in clinical laboratories; and (c) deal with applications of commercially prepared tests or kits to assays performed in clinical laboratories to measure the activities of established antimicrobial agents or their concentrations in body fluids are considered more appropriate for the *Journal of Clinical Microbiology*®. Manuscripts concerned with the development or modification of assay methods (e.g., high-throughput screening techniques) and validation of their sensitivity and specificity with a sufficiently large number of determinations or compounds are considered appropriate for AAC. Assay methods for the detection and characterization of concentrations of antimicrobials in sera or other body fluids are not within AAC's scope.

(iii) Susceptibility studies describing novel findings or testing new agents, and those with broad geographic reach, detailed mechanistic analysis, and important epidemiological implications, will be given higher priority than those testing isolates from local regions, with limited analysis, or with modest numbers of tested species or microorganisms. Single-center epidemiological studies (such as those defining risk factors for resistant infections) for which results are neither novel nor generalizable beyond the local environment are not appropriate for AAC.

(iv) Manuscripts describing new or novel methods or improvements in media and culture conditions will not be considered for publication in AAC unless these methods are applied to the study of problems related to the production or activity of antimicrobial agents. Such manuscripts are more appropriate for *Applied and Environmental Microbiology*® or the *Journal of Clinical Microbiology*®.

(v) Manuscripts dealing with properties of unpurified natural products, with entities that are primarily antitumor agents, or with immunomodulatory agents that are not antimicrobial agents are not appropriate for AAC. In addition, papers addressing photodynamic therapy are no longer appropriate for AAC.

(vi) Manuscripts dealing with novel small molecular antimicrobials must provide at least some data showing that the proposed new agents or scaffolds have the potential to become therapeutic agents. Appropriate demonstrations will vary but generally should be some combination of data on physical properties (solubility, protein binding, log *P* [logarithm of the ratio of the concentrations of un-ionized solutes in solvents]), pharmacological properties (Caco2 predictions of bioavailability, pharmacokinetics in an animal species), or tolerability (mammalian cell toxicity, likelihood of hepatic metabolism, potential for receptor interactions, potential for human ERG liability). Studies focusing on detailed mechanisms of cellular toxicity that lack whole-organ or animal studies are more appropriate for *Molecular and Cellular Biology* than for AAC. Initial presentations of compounds are not expected to address all these areas but rather to show an appropriate initial subset. For example, the first publication of a novel compound or compound series might address selected physical properties plus mammalian cell toxicity. Subsequent publications are expected to add progressively to the proof of the agent's therapeutic potential.

(vii) Biochemical analyses for β-lactamases that determine kinetic parameters (e.g., K_m, k_{cat}) must be performed on purified enzyme preparations. The enzyme must be in its native form, without any leader sequences or fusions used for purification (e.g., His tag). Enzymes for which the His tag has been removed can be considered native enzymes. The determination of relative rates of hydrolysis may be performed on crude extracts.

(viii) Authors of papers describing enzymological studies should review the standards of the STRENDA Commission for information required for adequate description of experimental conditions and for reporting enzyme activity data (http://www.beilstein-institut.de/en/projects/strenda/guidelines).

(ix) A manuscript limited to the nucleic acid sequence of a gene encoding an antibiotic target, receptor, or resistance mechanism may be submitted as a Short-Form paper (see "Short-Form Papers") or a New-Data Letter to the Editor (see "Letters to the Editor"), depending on its length. Formatting instructions for nucleic acid sequences are given below (see "Presentation of Nucleic Acid Sequences"). Repetition of sequences already in a database should be avoided.

Instructions to Authors are updated throughout the year. The current version is available on the journal website.

Questions about these guidelines may be directed to the editor in chief of the journal being considered.

If transfer to another ASM journal is recommended by an editor, the corresponding author will be contacted.

Note that a manuscript rejected by one ASM journal on scientific grounds or on the basis of its general suitability for publication is considered rejected by all other ASM journals.

EDITORIAL POLICY AND ETHICAL GUIDELINES

As a member of the Committee on Publication Ethics (COPE), ASM adheres to COPE's Best Practice Guidelines and expects authors to observe the high standards of publication ethics set out by COPE. ASM requirements for submitted manuscripts are consistent with the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, as last updated by the International Committee of Medical Journal Editors in December 2014 (http://www.icmje.org/).

Authors are expected to adhere to the highest ethical standards. The following sections of these Instructions include detailed information about ASM's ethical standards. Failure to comply with the policies described in these Instructions may result in a letter of reprimand, a suspension of publishing privileges in ASM journals, and/or notification of the authors' institutions. Authors employed by companies whose policies do not permit them to comply with ASM policies may be sanctioned as individuals and/or ASM may refuse to consider manuscripts having authors from such companies.

Use of Microbiological Information

The Council on Microbial Sciences (COMS) of the American Society for Microbiology affirms the long-standing position of the Society that microbiologists will work for the proper and beneficent application of science and will call to the attention of the public or the appropriate authorities misuses of microbiology or of information derived from microbiology. ASM members are obligated to discourage any use of microbiology contrary to the welfare of humankind, including the use of microbes as biological weapons. Bioterrorism violates the fundamental principles expressed in the Code of Ethics of the Society and is abhorrent to ASM and its members.

ASM recognizes that there are valid concerns regarding the publication of information in scientific journals that could be put to inappropriate use as described in the COMS resolution mentioned above. Members of the ASM Journals Board will evaluate the rare manuscript that might raise such issues during the review process. However, as indicated elsewhere in these Instructions, primary-research articles must contain sufficient detail, and material/information must be made available, to permit the work to be repeated by others. Supply of materials should be in accordance with laws and regulations governing the shipment, transfer, possession, and use of biological materials and must be for legitimate, bona fide research needs. We ask that authors pay particular attention to the NSAR Select Agent/Toxin list on the CDC website https://www.selectagents.gov/index.html and the U.S. Government Policy for Oversight of Life Sciences Dual Use Research of Concern (March 2012; http://www.phe.gov/s3/dualuse/Documents/us-policy-durc-032812.pdf).

Use of Human Subjects or Animals in Research

Authors of manuscripts describing research involving human subjects or animal experimentation must obtain review and approval (or review and waiver) from their Institutional Review Board (IRB) or Institutional Animal Care and Use Committee (IACUC), as appropriate, prior to manuscript submission. Authors of manuscripts that describe multisite research must obtain approval from each institution's IRB or IACUC, as appropriate. Documentation of IRB or IACUC status must be made available upon request. In the event that institutional review boards or committees do not exist, the authors must ensure that their research is carried out in accordance with the Declaration of Helsinki, as revised in 2013 (https://jamanetwork.com/journals/jama/fullarticle/1760318), and/or the "International Guiding Principles for Biomedical Research Involving Animals," as revised by the International Council for Laboratory Animal Science (ICLAS) and the Councils for International Organizations of Medical Sciences (CIOMS) in 2012. A statement of IRB or IACUC approval or waiver (and reason for waiver) or a statement of adherence to the Declaration of Helsinki and/or Guiding Principles must be included in the Materials and Methods section. The sex of research subjects and animals, and of materials derived directly from them (e.g., primary cell lines and clinical samples), should be included in the Materials and Methods section or Results section if these data are available.

Patient Identification

Informed consent is not needed if the patient cannot be identified from any material in a manuscript. In the absence of informed consent, identifying details, such as patient initials, specific dates, specific geographic exposures, or other identifying features (including body features in figures), should be omitted, but this must not alter the scientific meaning. Important information that is relevant to the scientific meaning should be stated so that the patient cannot be identified, e.g., by stating a season instead of a date or a region instead of a city. If a patient can be identified from the material in a manuscript, all efforts should be made to obtain informed consent to publish from patients or parents/legal guardians of minors. Informed consent requires that the patient have the opportunity to see the manuscript prior to submission. The written consent must state either that the patient has seen the complete manuscript or that the patient declines to do so. Patient consent should be archived with the authors and be available upon request. A statement attesting the receipt and archiving of written patient consent should be included in the published article.

Publishing Ethics

Authorship. ASM journals follow the criteria for authorship as outlined in the International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting,

Editing, and Publication of Scholarly Work in Medical Journals ("Defining the Role of Authors and Contributors"). Briefly, an author is one who makes a substantial contribution to the design, execution, and/or analysis and interpretation of experiments in addition to drafting, revising, and/or approving the initial submission and any subsequent versions of the article. All authors of a manuscript must have agreed to its submission and are responsible for appropriate portions of its content. Submission of a paper before all coauthors have read and approved it is considered an ethical violation.

Author contribution statements. As explained in the ICMJE recommendations, all persons designated as authors should qualify for authorship, and all those who qualify should be listed. ASM encourages transparency in authorship by publishing author contribution statements. Authors are strongly encouraged to include such statements in the Acknowledgments section.

Corresponding author. The corresponding author takes primary responsibility for communicating with the journal and coauthors throughout the submission, peer review, and publication processes. The corresponding author is responsible for ensuring that all coauthors have read and approved submissions, including appropriate citations, acknowledgments, and byline order. Additionally, the corresponding author and the study's primary investigator(s), if different, are required to have examined the raw data represented in the manuscript, affirm that such representations accurately reflect the original data, and ensure that the original data are preserved and retrievable.

Consortium authorship. A study group, surveillance team, working group, consortium, or the like (e.g., the Active Bacterial Core Surveillance Team) may be listed as a coauthor in the byline if its contributing members satisfy the requirements for authorship and accountability as described in these Instructions. The names (and institutional affiliations, if desired) of the contributing members only may be given as a separate paragraph in the Acknowledgments section. If the contributing members of the group associated with the work do not fulfill the criteria of substantial contribution to and responsibility for the paper, the group may not be listed in the author byline. Instead, it and the names of its contributing members may be listed in the Acknowledgments section.

Professional writers. "Ghost authorship" is not permitted by ASM. Professional writers should be mentioned in the Acknowledgments section rather than be included in the byline. To avoid perceived conflicts of interest, writer affiliations and specific contributions (for example, writing assistance, technical editing, language editing, or proofreading) must be disclosed.

Nonauthor contributions. Contributions from individuals who do not meet the ICMJE criteria for authorship should be acknowledged in the Acknowledgments section. Those that provided assistance, e.g., supplied strains or reagents or critiqued the paper, should not be listed as authors. Acquisition of funding, data collection, or general supervision of the research group does not qualify a person or persons for authorship. As mentioned above, professional writers do not meet authorship criteria and should be mentioned in the Acknowledgments section. Specific contributions for each nonauthor contributor should be included.

Byline order and changes. All authors must agree to the order in which their names are listed in the byline. Statements regarding equal contributions by two or more authors (e.g., "C.J. and Y.S. contributed equally to . . .") are permitted as footnotes to bylines and must be agreed to by all of the authors. A change in authorship (order of listing, addition or deletion of a name, or corresponding author designation) after submission of the manuscript will be implemented only after receipt of signed statements of agreement from all parties involved.

Authorship disputes. Disputes about authorship may delay or prevent review and/or publication of the manuscript. Should the individuals involved be unable to reach an accord, review and/or publication of the manuscript can proceed only after the matter is investigated and resolved by the authors' institution(s) and an official report provided to ASM. ASM does not itself investigate or attempt to resolve authorship disputes but will follow institutional recommendations, as appropriate.

ORCID. ASM Journals is a member of Open Researcher and Contributor ID (ORCID) and publishes author ORCID numbers in articles. ORCID is an open, nonprofit, community-driven effort to create and maintain a registry of unique researcher identifiers; it is a transparent method of linking research activities and output to these identifiers. In the eJournalPress (eJP) submission system, authors are encouraged to use or create an ORCID number, which can be linked to manuscripts and publications for which a researcher serves as an author. This can be helpful in distinguishing authors with common names. Additional information about ORCID is available on ORCID's website.

Plagiarism. Misappropriating another person's intellectual property constitutes plagiarism. This includes copying sentences or paragraphs verbatim (or almost verbatim) from someone else's work, even if the original work is cited in the references. The NIH Office of Research Integrity publication "Avoiding Plagiarism, Self-Plagiarism, and Other Questionable Writing Practices: a Guide to Ethical Writing" (https://ori.hhs.gov/avoiding-plagiarism-self-plagiarism-and-other-questionable-writing-practices-guide-ethical-writing) can help authors identify questionable writing practices.

Plagiarism is not limited to the text; it can involve any part of the manuscript, including figures and tables, in which material is copied from another publication without permission and attribution. An author may not reuse his or her own previously published work without attribution; this is considered text recycling (also known as self-plagiarism).

ASM has incorporated plagiarism detection software into its online submission and peer review system in order to help editors verify the originality of submitted manuscripts. Selected manuscripts are scanned and compared with databases. If plagiarism is detected, COPE guidelines on plagiarism will be followed.

Image manipulation. Submitted figures must reflect original data. Please refer to the "Image manipulation" section in Illustrations and Tables for an overview of permissible manipulations, unacceptable adjustments, and required information to be disclosed in the figure legends of images.

ASM applies forensic imaging tools to screen selected manuscripts for inappropriate manipulation of figures. If unacknowledged and/or inappropriate image manipulations are detected, the matter will be referred to the journal's ethics panel for consideration.

Fabrication, manipulation, and falsification of data. As a member of the Committee on Publication Ethics (COPE), ASM encourages authors to consult COPE's "Code of Conduct and Best Practice Guidelines for Journal Editors" (https://publicationethics.org/files/Code_of_conduct_for_journal_editors_0.pdf). Fabrication, manipulation, and falsification of data constitute misconduct. As defined by the U.S. Department of Health and Human Services, fabrication is "making up data or results and recording or reporting them," and falsification is "manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record" (42 Code of Federal Regulations, §93.103). All sources and methods used to obtain and analyze data, including any electronic preprocessing, should be fully disclosed; detailed explanations should be provided for any exclusions.

Primary publication. Manuscripts submitted to the journal must represent reports of original research, and the original data must be available for review by the editor if necessary. By submitting a manuscript to the journal, **the authors guarantee that they have the authority to publish the work and that the manuscript, or one with substantially the same content, was not published previously, is not being considered or published elsewhere, and was not rejected on scientific grounds by another ASM journal.** It is incumbent upon the author to acknowledge any prior publication, including his/her own articles, of the data contained in a manuscript submitted to an ASM journal. A copy of the relevant work should be submitted with the paper as supplemental material not for publication. Whether the material constitutes the substance of a paper and therefore renders the manuscript unacceptable for publication is an editorial decision.

In the event that the authors' previously published figures and/or data are included in a submitted manuscript, it is incumbent upon the corresponding author to (i) identify the duplicated material and acknowledge the source on the submission form, (ii) obtain permission from the original publisher (i.e., copyright owner), (iii) acknowledge the duplication in the figure legend, and (iv) cite the original article.

A paper is not acceptable for submission to an ASM journal if it, or its substance, has been made publicly available in the following:

- A serial, periodical, or book
- A conference report or symposium proceedings
- A technical bulletin or company white paper
- A public website (see "Preprint policy")
- Any other retrievable source

The following do not preclude submission to, or publication by, an ASM journal:

- Posting of a method/protocol on a public website
- Posting of a limited amount of original data on a personal/university/corporate website or websites of small collaborative groups working on a problem
- Deposit of unpublished sequence data in a public database
- Preliminary disclosures of research findings as meeting posters, webcast as meeting presentations, or published in abstract form as adjuncts to a meeting, e.g., part of a program
- Posting of theses and dissertations on a personal/university-hosted website

Preprint policy. ASM Journals will consider for publication manuscripts that have been posted in a recognized not-for-profit preprint archive provided that upon acceptance of the manuscript for publication, the author is still able to grant ASM copyright or agree to the terms of an Open Access license and pay the associated fee. It is the responsibility of authors to inform the journal at the time of submission if and where their article has been previously posted, and if the manuscript is accepted for publication in an ASM journal, authors are required to update the preprint with a citation to the final published article that includes the URL along with a link.

Conflict of Interest

All authors are expected to disclose, in the manuscript submittal letter, any commercial affiliations as well as consultancies, stock or equity interests, and patent-licensing arrangements that could be considered to pose a conflict of interest regarding the submitted manuscript. (Inclusion of a company name in the author address lines of the manuscript does not constitute disclosure.) Details of the disclosure to the editor will remain confidential. However, it is the responsibility of authors to provide, in the Acknowledgments section, a general statement disclosing conflicting interests relevant to the study. Examples of potentially conflicting interests include relationships, financial or otherwise, that might detract from an author's objectivity in presentation of study results and interests whose value would be enhanced by the results presented. All funding sources for the project, institutional and corporate, should be credited in the Acknowledgments section, as described below. In addition, if a manuscript concerns a commercial product, the manufacturer's name must be indicated in the Materials and Methods section or elsewhere in the text, as appropriate, in an obvious manner.

Data and Materials

Availability of data and materials. By publishing in the journal, the authors agree that, subject to requirements or limitations imposed by local and/or U.S. Government laws and regulations, any materials and data that are reasonably requested by others are available from a publicly accessible collection or will be made available in a timely fashion, at reasonable cost, and in limited quantities to members of the scientific

community for noncommercial purposes. Similarly, the authors agree to make available computer programs and/or code, originating in the authors' laboratory, that is the only means of confirming the conclusions reported in the article but that is not available commercially. The program(s) and suitable documentation regarding its (their) use may be provided by any of the following means: (i) as a program transmitted via the Internet, (ii) as an Internet server-based tool, or (iii) as a compiled or assembled form on a suitable medium. The authors guarantee that they have the authority to comply with this policy either directly or by means of material transfer agreements through the owner. ASM asks authors to assert this in a "Data availability" paragraph, which should appear at the end of the Materials and Methods section (or at the end of the text) of their submitted manuscript.

Data citation. To promote reproducibility, ASM expects researchers to identify and cite data sets and/or code used in their experiments and studies. These may be large or complex data sets that can include, but are not limited to, data from microarray, genomic, structural, proteomic, or video imaging analyses. **Authors should cite both the data set repository and the published article in which the data set and/or code was originally described.** Citations of data should be included in the reference list with persistent unique identifiers (e.g., active URLs, accession numbers, etc.). If computer code or software was created to generate results or interpret data, then a statement to that effect should be included in the "Data availability" paragraph. For cases in which the software is publicly available (e.g., FigTree to generate phylogenetic trees), the URL of the software informational page should be provided. **It is preferred that authors use established, publicly available data type-specific repositories.** If there is no appropriate repository available, general publicly available repositories should be used (e.g., Dryad, figshare, etc.). Examples of proper data citation are included in the "References" section of these Instructions to Authors.

Culture deposition. AAC expects authors to deposit strains used in therapeutic activity assessments and studies of mechanisms of action, resistance, and cross-resistance in publicly accessible culture collections and to refer to the collections and strain numbers in the text. Since the authenticity of subcultures of culture collection specimens that are distributed by individuals cannot be ensured, authors should indicate laboratory strain designations and donor sources as well as original culture collection identification numbers.

Authentication of cell lines. Cell line misidentification or contamination can adversely impact the validity of research findings. Authors should describe the source along with the date and method used for authentication of any cell lines used in manuscripts submitted to this journal. Cell lines used less than 6 months after receipt from a cell bank that performs authentication do not require reauthentication, but the source and method of authentication should be reported in the Materials and Methods section.

Nucleotide and amino acid sequences. Newly determined nucleotide and/or amino acid sequence data must be deposited and GenBank/ENA/DDBJ accession numbers must be included in the manuscript no later than the modification stage of the review process. It is expected that the sequence data will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged to comply with community metadata standards, such as the "Minimal Information about any (X) Sequence" (MIxS) checklist (http://gensc.org/projects/mixs-gsc-project/), when submitting to GenBank, ENA, or DDBJ. The accession numbers should be included in a separate paragraph with the lead-in "Accession number(s)" at the end of the Materials and Methods section for Research Articles or at the end of the text for Short-Form papers. If conclusions in a manuscript are based on the analysis of sequences and a GenBank/ENA/DDBJ accession number is not provided at the time of the review, authors should provide the annotated sequence data as supplemental material not for publication.

It is expected that, when previously published sequence accession numbers are cited in a manuscript, the original published article(s), as well as a citation of the database where the accession number is deposited, will be included in the References section.

Authors are also expected to do elementary searches and comparisons of nucleotide and amino acid sequences against the sequences in standard databases (e.g., GenBank) immediately before manuscripts are submitted and again at the proof stage.

Analyses should specify the database, and the date of each analysis should be indicated as, e.g., 6 January 2018. If relevant, the version of the software used should be specified.

See "Presentation of Nucleic Acid Sequences" for nucleic acid sequence formatting instructions.

The URLs of the databases mentioned above are as follows: DNA Data Bank of Japan (DDBJ), http://www.ddbj.nig.ac.jp/; European Nucleotide Archive (ENA), https://www.ebi.ac.uk/ena/; and GenBank, National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/nucleotide.

Proper use of locus tags as systematic identifiers for genes. To comply with recommendations from the International Nucleotide Sequence Database (INSD) Collaborators and to avoid conflicts in gene identification, researchers should implement the following two fundamental guidelines as standards for utilization of locus tags in genome analysis, annotation, submission, reporting, and publication. (i) Locus tag prefixes are systematic gene identifiers for all of the replicons of a genome and as such should be associated with a single genome project submission. (ii) New genome projects must be registered with the INSD, and new locus tag prefixes must be assigned in cooperation with the INSD to ensure that they conform to the agreed-upon criteria.

Structural determinations. Coordinates for new structures of macromolecules determined by X-ray crystallography or cryo-electron microscopy must be deposited in the Protein Data Bank and assigned identification codes must be included in the manuscript no later than the modification stage of the review process. It is expected that the coordinates will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged

to send coordinates with their original submission, however, so that reviewers can examine them along with the manuscript. The accession number(s) should be listed in a separate paragraph with the lead-in "Accession number(s)" at the end of the Materials and Methods section for Research Articles or at the end of the text for Short-Form papers.

The URLs for coordinate deposition are https://deposit-1.wwpdb.org/deposition/ and http://www.rcsb.org/pdb/home/home.do#Category-deposit.

Gene expression data. The entire set of supporting microarray, next-generation sequencing, or other high-throughput functional genomics data must be deposited in the appropriate public database (e.g., GEO, ArrayExpress, or CIBEX) and the assigned accession number(s) must be included in the manuscript no later than the modification stage of the review process. It is expected that the data will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged to send the relevant data with their original submission, however, so that reviewers can examine them along with the manuscript. The accession number(s) should be listed in a separate paragraph with the lead-in "Accession number(s)" at the end of the Materials and Methods section for Research Articles or at the end of the text for Short-Form papers.

The URLs of the databases mentioned above are as follows: Gene Expression Omnibus (GEO), https://www.ncbi.nlm.nih.gov/geo/; ArrayExpress, https://www.ebi.ac.uk/arrayexpress/; and Center for Information Biology Gene Expression Database (CIBEX), https://cibex.nig.ac.jp/data/index.html.

MycoBank. New scientific names of fungi along with key nomenclatural and descriptive material must be deposited in MycoBank (http://www.mycobank.org/) and the assigned accession number(s) must be included in the manuscript no later than the modification stage of the review process. It is expected that the data will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged to send the relevant data with their original submission, however, so that reviewers can examine them along with the manuscript. The accession number(s) should be listed in a separate paragraph with the lead-in "Accession number(s)" at the end of the Materials and Methods section for Research Articles and at the end of the text for Short-Form papers.

Copyright

For authors who do not opt to publish their papers as open access, ASM requires the corresponding author to sign a copyright transfer agreement on behalf of all the authors.

In the copyright transfer agreement signed by an author, ASM grants to that author (and coauthors) the right to republish discrete portions of his/her (their) article in any other publication (print, CD-ROM, and other electronic forms) of which he/she is (they are) the author(s) or editor(s), on the condition that appropriate credit is given to the original ASM publication. This republication right also extends to posting on a host computer to which there is access via the Internet. Except as indicated below, significant portions of the article may not be reprinted/posted without ASM's prior written permission, however, as this would constitute duplicate publication.

Authors may post their own published articles on their personal or university-hosted (but not corporate, government, or similar) websites without ASM's prior written permission provided that appropriate credit is given (i.e., the copyright lines shown at the bottom of the first page of the PDF version).

Works authored solely by U.S. Government employees are not subject to copyright protection, so there is no copyright to be transferred. However, the other provisions of the copyright transfer agreement, such as author representations of originality and authority to enter into the agreement, apply to U.S. Government employee authors as well as to other authors.

When funds from the Wellcome Trust, Research Councils UK, or the Bill and Melinda Gates Foundation are used to pay an article open access fee, the article will be published under the Creative Commons Attribution 4.0 International license (CC BY 4.0) in accordance with the funding organization's open access policies. Authors will be required to notify ASM and complete the Author Warranty and Provisional License to Publish at the time of submission.

Copyright for supplemental material (see "Supplemental Material") remains with the author, but a license permitting the posting by ASM is included in the article copyright transfer agreement. If the author of the article is not also the copyright owner of the supplemental material, the corresponding author must send to ASM signed permission from the owner that allows posting of the material, as a supplement to the article, by ASM. The corresponding author is also responsible for incorporating into the supplemental material any copyright notices required by the owner.

Permissions

The corresponding author is responsible for obtaining permission from both the original author and the original publisher (i.e., the copyright owner) to reproduce or modify figures and tables and to reproduce text (in whole or in part) from previous publications.

Permission(s) must be obtained no later than the modification stage. The original signed permission(s) must be identified as to the relevant item in the ASM manuscript (e.g., "permissions for Fig. 1 in AAC00123-18") and submitted to the ASM production editor on request. In addition, a statement indicating that the material is being reprinted with permission must be included in the relevant figure legend or table footnote of the manuscript. Reprinted text must be enclosed in quotation marks, and the permission statement must be included as running text or indicated parenthetically.

It is expected that the authors will provide written assurance that permission to cite unpublished data or personal communications has been granted.

For supplemental material intended for posting by ASM (see "Supplemental Material"), if the authors of the AAC manuscript are not also the owners of the supplemental material, the corresponding author must send to ASM signed permission from the copyright owner that allows posting of the material, as a supplement to the article, by ASM. The corresponding

author is also responsible for incorporating in the supplemental material any copyright notices required by the owner.

Warranties and Exclusions

Articles published in this journal represent the opinions of the authors and do not necessarily represent the opinions of ASM. ASM does not warrant the fitness or suitability, for any purpose, of any methodology, kit, product, or device described or identified in an article. The use of trade names is for identification purposes only and does not constitute endorsement by ASM.

SUBMISSION, REVIEW, AND PUBLICATION PROCESSES

Submission Process

All submissions to AAC must be made electronically via the eJournalPress (eJP) online submission and peer review system at the following URL: https://aac.msubmit.net/cgi-bin/main.plex. (E-mailed submissions will not be accepted.) First-time users must create an Author account, which may be used for submitting to all ASM journals. Instructions for creating an Author account are available at the above URL via the "help for authors" link, and step-by-step instructions for submitting a manuscript via eJP are also available through the same link on the log-in screen or on the account holder's Home page. Information on file types acceptable for electronic submission can be found under the Files heading in the help for authors screen.

Review Process

All manuscripts are considered to be confidential and are reviewed by the editors, members of the editorial board, or qualified *ad hoc* reviewers. To expedite the review process, authors must recommend at least three reviewers who have expertise in the field, who are not members of their institution(s), who have not recently been associated with their laboratory(ies), and who could not otherwise be considered to pose a conflict of interest regarding the submitted manuscript. Impersonation of another individual during the review process is considered serious misconduct. At least one recommended reviewer must be a member of the journal's editorial board. Please provide, where indicated on the submission form, contact information for suggested reviewers who are not editorial board members.

To facilitate the review, copies of in-press and submitted manuscripts that are important for judgment of the present manuscript should be included as supplemental material not for publication.

When a manuscript is submitted to the journal, it is given a control number (e.g., AAC00123-18) and assigned to one of the editors. (**Always refer to this control number in communications with the editor and the Journals Department.**) From there it is assigned to at least two independent experts for peer review. A single-blind review, where authors' identities are known to reviewers, is applied. It is the responsibility of the corresponding author to inform the coauthors of the manuscript's status throughout the submission, review, and publication processes. The reviewers operate under strict guidelines set forth in "Guidelines for Reviewers" (http://www.journals.asm.org/site/misc/reviewguide.xhtml) and are expected to complete their reviews expeditiously.

The corresponding author is notified, generally within 4 to 6 weeks after submission, of the editor's decision to accept, reject, or require modification. When modification is requested, the corresponding author must either submit the modified version within 2 months or withdraw the manuscript. A point-by-point response to the reviews must be uploaded as a separate file (identified as such), and a compare copy of the manuscript (without figures) should be included as a Marked Up Manuscript.

Manuscripts that have been rejected with the option to resubmit, or withdrawn after being returned for modification, may be resubmitted to the same ASM journal if the major criticisms have been addressed. A manuscript rejected on scientific grounds or on the basis of its general suitability for publication by one ASM journal, with the exception of *mBio*®, is considered rejected by all other ASM journals. A rejection from *mBio* does not disqualify a manuscript from being newly submitted to another ASM journal (the rejection by *mBio* need not be mentioned in the cover letter). A manuscript rejected solely on the basis of scope may be resubmitted to a more appropriate ASM journal.

The cover letter for every resubmitted manuscript must state that the manuscript is a resubmission, and the former manuscript control number must be provided. A point-by-point response to the review(s) must be uploaded as a separate file (identified as such), and a copy of the revised manuscript tracking the changes must be included as a Marked Up Manuscript. Manuscripts resubmitted to the same journal are normally handled by the original editor. Manuscripts rejected with the option to resubmit may be resubmitted only once unless permission has been obtained from the original editor or from the editor in chief.

Notification of Acceptance

When an editor has decided that a manuscript is acceptable for publication on the basis of scientific merit, the author and the Journals Department are notified. A PDF version of the accepted manuscript is posted online as soon as possible (see "AAC Accepts").

The text files undergo an automated preediting, cleanup, and tagging process specific to the particular article type, and the illustrations are examined. If all files have been prepared according to the criteria set forth in these Instructions and those in the eJP online manuscript submission system, the acceptance procedure will be completed successfully. If there are problems that would cause extensive corrections to be made at the copyediting stage or if the files are not acceptable for production, ASM Journals staff will contact the corresponding author. Once all the material intended for publication has been determined to be adequate, the manuscript is scheduled for the next available issue. The editorial staff of the ASM Journals Department completes the editing of the manuscript to bring it into conformity with prescribed standards.

AAC Accepts

For its primary-research journals, ASM posts online PDF versions of manuscripts that have been peer reviewed and accepted but not yet copyedited. This feature is called "[journal acronym] Accepts" (e.g., AAC Accepts). The manuscripts are published online as soon as possible after acceptance, on a weekly basis, before the copyedited, typeset articles are published. They are posted "as is" (i.e., as submitted by the authors at the modification stage) and do not reflect ASM editorial changes. No corrections/changes to the PDF manuscripts are accepted. Accordingly, there likely will be differences between the AAC Accepts manuscripts and the final, typeset articles. The manuscripts remain listed on the AAC Accepts page until the final, typeset articles are posted. At that point, the manuscripts are removed from the AAC Accepts page. The manuscripts are under subscription access control until 6 months after the typeset articles are posted, when free access is provided to everyone (subject to the applicable ASM license terms and conditions). Supplemental material intended, and accepted, for publication is not posted until publication of the final, typeset article.

The ASM embargo policy allows a press release to be issued as soon as the accepted manuscript is posted on the AAC Accepts page. To be notified as soon as your manuscript is posted, please sign up for e-Alerts at http://aac.asm.org/cgi/alerts.

Instructions on how to cite such manuscripts may be found in "References."

Page Proofs

Page proofs, together with a query sheet and instructions for handling proofs, will be made available to the corresponding author electronically. Queries must be answered on the query page, and any changes related to the queries, as well as any additional changes, must be indicated on the proofs. Note that the copy editor does not query at every instance where a change has been made. Queries are written only to request necessary information or clarification of an unclear passage or to draw attention to edits that may have altered the sense. It is the author's responsibility to read the entire text, tables, and figure legends, not just items queried. Corrected proofs must be returned within two business days after notification of availability.

The proof stage is not the time to make extensive corrections, additions, or deletions. Figures as they appear in the proofs are for validation of content and placement, not quality of reproduction or color accuracy. Print output of figures in the PDF page proofs will be of lower quality than the same figures viewed on a monitor. Please avoid making changes to figures based on quality of color or reproduction in proof.

Important new information that has become available between acceptance of the manuscript and receipt of the proofs may be inserted as an addendum in proof with the permission of the editor. If references to unpublished data or personal communications are added, it is expected that written assurance granting permission for the citation will be included. Limit changes to corrections of spelling errors, incorrect data, and grammatical errors and updated information for references to articles that have been submitted or are in press. If URLs have been provided in the article, recheck the sites to ensure that the addresses are still accurate and the material that you expect the reader to find is indeed there.

Questions about proofs should be directed to the ASM Journals Department (e-mail, nlin@asmusa.org; telephone, 202-942-9231).

PDF Files

The corresponding author will have limited access (10 downloads, total) to the PDF file of his/her published article. An e-mail alert will automatically be sent to him/her on the day the issue is posted. It will provide a URL, which will be required to obtain access, and instructions. An article may be viewed, printed, or stored, provided that it is for the author's own use.

Should coauthors or colleagues be interested in viewing the paper for their own use, the corresponding author may provide them with the URL; a copy of the article may not be forwarded electronically. However, they must be made aware of the terms and conditions of the ASM copyright. (For details, go to http://www.journals.asm.org/site/misc/terms.xhtml.) Note that each such download will count toward the corresponding author's total of 10. After 10 downloads, access will be denied and can be obtained only through a subscription to the journal (either individual or institutional) or after the standard access control has been lifted (i.e., 6 months after publication).

Funding Agency Repositories

The National Institutes of Health (NIH) requests that its grantee and intramural authors provide copies of their accepted manuscripts to PubMed Central (PMC) for posting in the PMC Public Access Repository. AAC authors are automatically in compliance with this policy and need take no action themselves. For the past several years, ASM has deposited in PubMed Central all publications from all ASM journals. Further, ASM policy is that all primary-research articles are made available to everyone, free, 6 months after publication through PubMed Central, HighWire, and international PubMed Central-like repositories. By having initiated these policies, ASM is in full compliance with NIH policy. For more information, see https://publicaccess.nih.gov/.

ASM also allows AAC authors whose work was supported by funding agencies that have public access requirements like those of the NIH (e.g., the Wellcome Trust) to post their accepted manuscripts in publicly accessible electronic repositories maintained by those funding agencies. If a funding agency does not itself maintain such a site, then ASM allows the author to fulfill that requirement by depositing the manuscript (not the typeset article) in an appropriate institutional or subject-based open repository established by a government or noncommercial entity.

Since ASM makes the final, typeset articles from its primary-research journals available free of charge on the ASM Journals and PMC websites 6 months after final publication, ASM requests that when submitting the accepted manuscript to PMC or a similar public access site, the author specify that the **posting release date for the manuscript be no earlier than 6 months after publication of the typeset article by ASM and that a link to the published manuscript on the journal website be provided.**

Publication Fees

APCs. Authors who choose open access will be assessed an article processing charge (APC). For a **corresponding author who is an active member of ASM at any level except the Supporting member level,** the APC is $2,300 (subject to change without notice). For a **nonmember or Supporting member corresponding author,** the APC is $3,150 (subject to change without notice). Nonmember corresponding authors may join ASM to obtain discounts on APCs. Former members who wish to renew their membership at the same level may do so online. However, to change your membership level, please contact customer service at Service@asmusa.org. These fees are in addition to any supplemental material charges and permit immediate public access to both the preliminary "Accepts" version and the copyedited, typeset version published in the online journal under the Creative Commons Attribution 4.0 International license (CC BY 4.0). This option includes immediate open access provided through NIH's PubMed Central repository.

When funds from the Wellcome Trust, Research Councils UK, or the Bill and Melinda Gates Foundation are used to pay an APC, the article will be published under the CC BY 4.0 in accordance with the funding organization's open access policies. Authors will be required to notify ASM and complete the Author Warranty and Provisional License to Publish/CC BY 4.0 at the time of submission.

Page charges. Authors who do not choose open access and whose research was supported by grants, special funds (including departmental and institutional), or contracts (including governmental) or whose research was done as part of their official duties (government or corporate, etc.) are required to pay page charges (based on the number of typeset pages, including illustrations, in the article) and to sign the ASM copyright transfer agreement. Corresponding authors of articles accepted for publication will receive an e-mail notifying them how to pay page and any other applicable publication charges (see below).

For a **corresponding author who is an active member of ASM at any level except the Supporting member level**, page charges are $80 per page (subject to change without notice).

For a **nonmember or Supporting member corresponding author**, page charges are $160 per page (subject to change without notice). Nonmember corresponding authors may join ASM to obtain discounts on publication fees. Former members who wish to renew their membership at the same level may do so online. However, to change your membership level, please contact customer service at Service@asmusa.org.

If the research was not supported by any of the means described above, a request to waive the charges may be sent to the ASM Journals Department (e-mail, nlin@asmusa.org [after acceptance of the manuscript]). The request must include the manuscript control number assigned by ASM and indicate how the work was supported. Waivers apply only to page charges; responsibility for supplemental material fees remains with the author.

Minireviews, Commentaries, and Comment Letters to the Editor are not subject to page charges. New-Data Letters to the Editor are subject to page charges.

Color charges. There are no fees for color figures.

Author reprints and eprints. Reprints (in multiples of 100) and eprints (downloadable PDFs) may be purchased by all coauthors. In addition to receiving the 10 free published PDF files mentioned above, the corresponding authors of Minireviews may receive 100 free eprints of their contribution and the corresponding authors of Commentaries may receive 50 free eprints. Instructions for ordering gratis or additional reprints and eprints can be found in the billing notification e-mail sent to all corresponding authors. To order reprints postpublication, please follow the instructions on the Author Reprint Order Form. Please contact cjsreprints@cadmus.com with any questions.

Supplemental material fee. Authors are charged a flat fee for posting supplemental material as an adjunct to their published article. (Exception: no fee is charged for supplemental material associated with Minireviews or Commentaries.)

For a **corresponding author who is an active member of ASM at any level except the Supporting member level**, the supplemental material fee is $210 (subject to change without notice). For a **nonmember or Supporting member corresponding author**, the supplemental material fee is $320 (subject to change without notice). Nonmember corresponding authors may join ASM to obtain discounts on publication fees. Former members who wish to renew their membership at the same level may do so online. However, to change your membership level, please contact customer service at Service@asmusa.org.

ORGANIZATION AND FORMAT

Editorial Style

The editorial style of ASM journals conforms to the *ASM Style Manual for Journals* (American Society for Microbiology, 2018, in-house document [you may find the ASM Word List helpful]) and *How To Write and Publish a Scientific Paper*, 7th ed. (Greenwood, Santa Barbara, CA, 2011), as interpreted and modified by the editors and the ASM Journals Department.

The editors and the Journals Department reserve the privilege of editing manuscripts to conform with the stylistic conventions set forth in the aforesaid publications and in these Instructions. Please note that ASM uses the serial comma.

On receipt at ASM, an accepted manuscript undergoes an automated preediting, cleanup, and tagging process specific to the particular article type. To optimize this process, manuscripts must be supplied in the correct format and with the appropriate sections and headings.

Type every portion of the manuscript double-spaced (a minimum of 6 mm between lines), including figure legends, table footnotes, and references, and number all pages in sequence, including the abstract, figure legends, and tables. Place the last two items after the References section. Manuscript pages must have continuous line numbers; manuscripts without line numbers may be editorially rejected by the editor, with a suggestion of resubmission after line numbers are added. The font size should be no smaller than 12 points. It is recommended that the following sets of characters be easily distinguishable in the manuscript: the numeral zero (0) and the let-

ter "oh" (O); the numeral one (1), the letter "el" (l), and the letter "eye" (I); and a multiplication sign (×) and the letter "ex" (x). Do not create symbols as graphics or use special fonts that are external to your word processing program; use the "insert symbol" function. Set the page size to 8.5 by 11 inches (ca. 21.6 by 28 cm). Italicize any words that should appear in italics, and indicate paragraph lead-ins in boldface type.

Manuscripts may be editorially rejected, without review, on the basis of poor English or lack of conformity to the standards set forth in these Instructions.

Authors who are unsure of proper English usage should have their manuscripts checked by someone proficient in the English language or engage a professional language editing service for help.

Manuscript Submission Checklist

- Double-space all text, including references and figure legends.
- Number pages.
- Number lines continuously.
- Present statistical treatment of data where appropriate.
- Provide accession numbers for all newly published sequences in a dedicated paragraph, and if a sequence or sequence alignment important for evaluation of the manuscript is not yet available, provide the information as supplemental material not for publication or make the material available on a website for access by the editor and reviewers.
- Format references in ASM style.
- Provide references for accession numbers and code (with URLs).
- Confirm that genetic and chemical nomenclature conforms to instructions.
- Include as supplemental material not for publication in-press and submitted manuscripts that are important for judgment of the present manuscript.

Supplemental Material

Supplemental material will be peer reviewed along with the manuscript and must be uploaded to the eJournalPress (eJP) peer review system at initial manuscript submission. The decision to publish the material online with the accepted article is made by the editor. It is possible that a manuscript will be accepted but that the supplemental material will not be.

All supplemental text, tables, and figures should be combined in a single self-contained document (PDF), and no supplemental material should be included in the main manuscript. Supplemental data set and movie files may be uploaded separately. The number of supplemental material files is limited to 10. Supplemental files should be submitted in the following standard formats.

- **Text, figures, tables, and legends** should be included in a single PDF file. All figures and tables should be numbered independently and cited at the relevant point in the manuscript text, e.g., "Fig. S1," "Fig. S2," "Table S3," etc. Do not duplicate data by presenting them in both the text of the manuscript and a supplemental figure. Each legend should appear below its corresponding figure or table. The maximum file size is 8 MB. Please review this sample file for guidance.
- **Data set** (Excel [.xls]) files should include a brief description of how the data are used in the paper. The maximum file size is 20 MB. Please review this sample file for guidance.
- **Movies** (Audio Video Interleave [.avi], QuickTime [.mov], or MPEG files) should be submitted at the desired reproduction size and length and should be accompanied by a legend. The maximum file size is 20 MB.

Unlike the manuscript, supplemental material will not be edited by the ASM Journals staff and proofs will not be made available. References related to supplemental material only should not be listed in the References section of an article; instead, include them with the supplemental material. Supplemental material will always remain associated with its article and is not subject to any modifications after publication.

Material that has been published previously (print or online) is not acceptable for posting as supplemental material. Instead, the appropriate reference(s) to the original publication should be made in the manuscript.

Copyright for the supplemental material remains with the author, but a license permitting posting by ASM is included in the copyright transfer agreement completed by the corresponding author. If you are not the copyright owner, you must provide to ASM signed permission from the owner that allows posting of the material, as a supplement to your article, by ASM. You are responsible for including in the supplemental material any copyright notices required by the owner.

See also "Publication Fees."

Research Articles

Research Articles should include the elements described in this section.

Title, running title, byline, affiliation line(s), and corresponding author. Each manuscript should present the results of an independent, cohesive study; thus, numbered series titles are not permitted. Exercise care in composing a title. Avoid the main title/subtitle arrangement, complete sentences, and unnecessary articles. On the title page, include the title, the running title (not to exceed 54 characters and spaces), the name of each author, all authors' affiliations at the time the work was performed, the name(s) and e-mail address(es) of the corresponding author(s), and a footnote indicating the present address of any author no longer at the institution where the work was performed. Place a number sign (#) in the byline after the affiliation letter(s) of the author to whom inquiries regarding the paper should be directed (see "Correspondent footnote," below). Indicate each author's affiliation with a superscript lowercase letter placed after the author's surname in the byline (separate multiple affiliation letters with commas but no space). Each affiliation should have its own line and its own superscript affiliation letter preceding it. Do not consolidate

different departments at one institution into one address with a single affiliation letter, even if all affected authors belong to all of those departments. Please review this sample title page for guidance.

Study group in byline. A study group, surveillance team, working group, consortium, or the like (e.g., the Active Bacterial Core Surveillance Team) may be listed as a coauthor in the byline if its contributing members satisfy the requirements for authorship and accountability as described in these Instructions. The names (and institutional affiliations if desired) of the contributing members may be given as a separate paragraph in Acknowledgments.

If the contributing members of the group associated with the work do not fulfill the criteria of substantial contribution to and responsibility for the paper, the group may not be listed in the author byline. Instead, it and the names of its contributing members may be listed in the Acknowledgments section.

Correspondent footnote. The e-mail address for the corresponding author should be included on the title page of the manuscript. This information will be published in the article as a footnote to facilitate communication and will be used to notify the corresponding author of the availability of proofs and, later, of the PDF file of the published article. No more than two authors may be designated corresponding authors.

Abstract. Limit the abstract to 250 words or fewer and concisely summarize the basic content of the paper without presenting extensive experimental details. Avoid abbreviations and references, and do not include diagrams. When it is essential to include a reference, use the format shown under "References" below (see the "Citations in abstracts" section). Conclude the abstract with a summary statement. Because the abstract will be published separately by abstracting services, it must be complete and understandable without reference to the text.

Introduction. The introduction should supply sufficient background information to allow the reader to understand and evaluate the results of the present study without referring to previous publications on the topic. The introduction should also provide the hypothesis that was addressed or the rationale for the study. References should be chosen carefully to provide the most salient background rather than an exhaustive review of the topic.

Results. In the Results section, include the rationale or design of the experiments as well as the results; reserve extensive interpretation of the results for the Discussion section. Present the results as concisely as possible in one of the following: text, table(s), or figure(s). Avoid extensive use of graphs to present data that might be more concisely or more quantitatively presented in the text or tables. Limit photographs (particularly photomicrographs and electron micrographs) to those that are absolutely necessary to show the experimental findings. Number figures and tables in the order in which they are cited in the text, and be sure that all figures and tables are cited.

Discussion. The Discussion should provide an interpretation of the results in relation to previously published work and to the experimental system at hand and should not contain extensive repetition of the Results section or reiteration of the introduction. In short papers, the Results and Discussion sections may be combined.

Materials and Methods. The Materials and Methods section should include sufficient technical information to allow the experiments to be repeated. When centrifugation conditions are critical, give enough information to enable another investigator to repeat the procedure: make of centrifuge, model of rotor, temperature, time at maximum speed, and centrifugal force ($\times g$ rather than revolutions per minute). For commonly used materials and methods (e.g., media and protein concentration determinations), a simple reference is sufficient. If several alternative methods are commonly used, it is helpful to identify the method briefly as well as to cite the reference. For example, it is preferable to state "cells were broken by ultrasonic treatment as previously described (9)" rather than "cells were broken as previously described (9)." This allows the reader to assess the method without constant reference to previous publications. Describe new methods completely, and give sources of unusual chemicals, equipment, or microbial strains. When large numbers of microbial strains or mutants are used in a study, include tables identifying the immediate sources (i.e., sources from whom the strains were obtained) and properties of the strains, mutants, bacteriophages, and plasmids, etc.

A method or strain, etc., used in only one of several experiments reported in the paper may be described in the Results section or very briefly (one or two sentences) in a table footnote or figure legend. It is expected that the sources from whom the strains were obtained will be identified.

As noted above, a paragraph dedicated to new accession numbers for nucleotide and amino acid sequences, microarray data, protein structures, gene expression data, and MycoBank data should appear at the end of Materials and Methods with the paragraph lead-in "Accession number(s)." Please also provide references (with URLs) for the accession numbers.

Acknowledgments. Statements regarding sources of direct financial support (e.g., grants, fellowships, and scholarships, etc.) should appear in the Acknowledgments. A funding statement indicating what role, if any, the funding agency had in your study (for example, "The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.") may be included. Funding agencies may have specific wording requirements, and compliance with such requirements is the responsibility of the author. In cases in which research is not funded by any specific project grant, funders need not be listed, and the following statement may be used: "This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors." Statements regarding indirect financial support (e.g., commercial affiliations, consultancies, stock or equity interests, and patent-licensing arrangements) are also allowed. It is the responsibility of authors to provide a general statement

disclosing financial or other relationships that are relevant to the study. (See the "Conflict of Interest" section above.)

Recognition of personal assistance should be given in the Acknowledgments section, as should any statements disclaiming endorsement or approval of the views reflected in the paper or of a product mentioned therein.

In addition to acknowledging sources of financial support in the manuscript, authors should list any sources of funding in response to the Funding Sources question on the online submission form, providing relevant grant numbers where possible, and the authors associated with the specific funding sources. In the event that your submission is accepted, the funding source information provided in the submission form may be published, so please ensure that all information is entered accurately and completely. (It will be assumed that the absence of any information in the Funding Sources fields is a statement by the authors that no support was received.)

Appendixes. Appendixes that contain additional material to aid the reader are permitted. Titles, authors, and reference sections that are distinct from those of the primary article are not allowed. If it is not feasible to list the author(s) of the appendix in the byline or the Acknowledgments section of the primary article, rewrite the appendix so that it can be considered for publication as an independent article, either full-length or Short-Form style. Equations, tables, and figures should be labeled with the letter "A" preceding the numeral to distinguish them from those cited in the main body of the text.

References. In the reference list, references are numbered in the order in which they are cited in the article (citation-sequence reference system). In the text, references are cited parenthetically by number in sequential order. Data that are not published or not peer reviewed are simply cited parenthetically in the text (see section ii below).

(i) References listed in the References section. The following types of references must be listed in the References section:

- Journal articles (both print and online)
- Books (both print and online)
- Book chapters (publication title is required)
- Patents
- Theses and dissertations
- Published conference proceedings
- Meeting abstracts (from published abstract books or journal supplements)
- Letters (to the editor)
- Company publications
- In-press journal articles, books, and book chapters
- Data sets
- Code

Provide the names of all the authors and/or editors for each reference; long bylines should not be abbreviated with "et al." All listed references must be cited in the text. Abbreviate journal names according to the PubMed Journals Database (National Library of Medicine, National Institutes of Health; available at https://www.ncbi.nlm.nih.gov/nlmcatalog/journals), the primary source for ASM style (do not use periods with abbreviated words). The EndNote output style for ASM Journals' current reference style can be found at http://journals.asm.org/site/misc/ASM_Journals.ens; click "Open" and then "Download and Install" to save it to your EndNote Styles folder (it should replace any earlier output styles for ASM journals [all ASM journals use the same reference style]). Note that DOIs are not needed for most references. ASM copy editors will automatically insert DOIs on all references in the CrossRef and PubMed databases during copyediting. URLs for government reports and other references not indexed in these databases should be provided if desired; URLs for citations of database accession numbers and code/software should be provided by you.

Follow the styles shown in the examples below.

1. Caserta E, Haemig HAH, Manias DA, Tomsic J, Grundy FJ, Henkin TM, Dunny GM. 2012. *In vivo* and *in vitro* analyses of regulation of the pheromone-responsive *prgQ* promoter by the PrgX pheromone receptor protein. J Bacteriol 194:3386–3394.
2. Bina XR, Taylor DL, Vikram A, Ante VM, Bina JE. 2013. *Vibrio cholerae* ToxR downregulates virulence factor production in response to cyclo(Phe-Pro). mBio 4:e00366-13.
3. Winnick S, Lucas DO, Hartman AL, Toll D. 2005. How do you improve compliance? Pediatrics 115:e718–e724.
4. Falagas ME, Kasiakou SK. 2006. Use of international units when dosing colistin will help decrease confusion related to various formulations of the drug around the world. Antimicrob Agents Chemother 50:2274–2275. (Letter.) {*"Letter" or "Letter to the editor" is allowed but not required at the end of such an entry.*}
5. Cox CS, Brown BR, Smith JC. J Gen Genet, in press.* {*Article title is optional; journal title is mandatory.*}
6. Forman MS, Valsamakis A. 2011. Specimen collection, transport, and processing: virology, p 1276–1288. *In* Versalovic J, Carroll KC, Jorgensen JH, Funke G, Landry ML, Warnock DW (ed), Manual of clinical microbiology, 10th ed, vol 2. ASM Press, Washington, DC.
7. da Costa MS, Nobre MF, Rainey FA. 2001. Genus I. Thermus Brock and Freeze 1969, 295,[AL] emend. Nobre, Trüper and da Costa 1996b, 605, p 404–414. *In* Boone DR, Castenholz RW, Garrity GM (ed), Bergey's manual of systematic bacteriology, 2nd ed, vol 1. Springer, New York, NY.
8. Fitzgerald G, Shaw D. *In* Waters AE (ed), Clinical microbiology, in press. EFH Publishing Co, Boston, MA.* {*Chapter title is optional.*}
9. Green PN, Hood D, Dow CS. 1984. Taxonomic status of some methylotrophic bacteria, p 251–254. *In* Crawford RL, Hanson RS (ed), Microbial growth on C_1 compounds. Proceedings of the 4th International Symposium. American Society for Microbiology, Washington, DC.
10. Rotimi VO, Salako NO, Mohaddas EM, Philip LP. 2005. Abstr 45th Intersci Conf Antimicrob Agents Chemother, abstr D-1658. {*Abstract title is optional.*}
11. Smith D, Johnson C, Maier M, Maurer JJ. 2005. Distribution of fimbrial, phage and plasmid associated virulence genes among poultry *Salmonella enterica* serovars, abstr P-038, p 445. Abstr 105th Gen Meet Am Soc Microbiol. American Society for Microbiology, Washington, DC. {*Abstract title is optional.*}

12. García CO, Paira S, Burgos R, Molina J, Molina JF, Calvo C, Vega L, Jara LJ, García-Kutzbach A, Cuellar ML, Espinoza LR. 1996. Detection of *Salmonella* DNA in synovial membrane and synovial fluid from Latin American patients using the polymerase chain reaction. Arthritis Rheum 39(Suppl 9):S185. {*Meeting abstract published in journal supplement.*}
13. O'Malley DR. 1998. PhD thesis. University of California, Los Angeles, CA. {*Title is optional.*}
14. Stratagene. 2006. Yeast DNA isolation system: instruction manual. Stratagene, La Jolla, CA. {*Use the company name as the author if none is provided for a company publication.*}
15. Odell JC. April 1970. Process for batch culturing. US patent 484,363,770. {*Include the name of the patented item/process if possible; the patent number is mandatory.*}
16. Harrison F, Roberts AEL, Gabrilska R, Rumbaugh KP, Lee C, Diggle SP. 2015. A 1,000-year-old antimicrobial remedy with antistaphylococcal activity. mBio 6:e01129-15. {*Original article that describes how data submitted to a database were generated.*}
17. Harrison F, Roberts AEL, Gabrilska R, Rumbaugh KP, Lee C, Diggle SP. 2015. Data from "A 1,000-year-old antimicrobial remedy with antistaphylococcal activity." Dryad Digital Repository https://doi.org/10.5061/dryad.mn17p. {*Citation for the database where the data in the previous reference were deposited; the URL is necessary.*}
18. Wang Y, Rozen D. 2016. Colonization and transmission of the gut microbiota of the burying beetle, *Nicrophorus vespilloides*, through development. bioRxiv https://doi.org/10.1101/091702.

*A reference to an in-press ASM publication should state the control number (e.g., AAC00123-18) if it is a journal article or the name of the publication if it is a book.

In some online journal articles, posting or revision dates may serve as the year of publication; a DOI (preferred) or URL is required for articles with nontraditional page numbers or electronic article identifiers.

Magalon A, Mendel RR. 15 June 2015, posting date. Biosynthesis and insertion of the molybdenum cofactor. EcoSal Plus 2015 doi:10.1128/ecosalplus.ESP-0006-2013.

Note: a posting or accession date is required for any online reference that is periodically updated or changed.

Citations of ASM Accepts manuscripts should look like the following example.

Wang GG, Pasillas MP, Kamps MP. 15 May 2006. Persistent transactivation by Meis1 replaces Hox function in myeloid leukemogenesis models: evidence for co-occupancy of Meis1-Pbx and Hox-Pbx complexes on promoters of leukemia-associated genes. Mol Cell Biol doi:10.1128/MCB.00586-06.

Other journals may use different styles for their publish-ahead-of-print manuscripts, but citation entries must include the following information: author name(s), posting date, title, journal title, and volume and page numbers and/or DOI. The following is an example:

Zhou FX, Merianos HJ, Brunger AT, Engelman DM. 13 February 2001. Polar residues drive association of polyleucine transmembrane helices. Proc Natl Acad Sci U S A doi:10.1073/pnas.041593698

To encourage data sharing and reuse, ASM recommends reporting data sets and/or code both in a dedicated "Data availability" paragraph and in References. The components of a complete data citation include the following:

- Responsible party (senior author, collector, agency),
- Publication year,
- Complete name of a data set, including the name of the database or repository and its URL, **or** the name of the analysis software (if appropriate), including the version and project,
- Publisher (if appropriate), and
- Persistent unique identifier(s) (e.g., URL[s] or accession number[s]).

The following templates may be helpful.

Author. Year. Description of study topic. Retrieved from Database URL (accession no. ●●●●●●). {*Unpublished raw data.*}

Author. Year. Description or title of software (version). Repository URL. Retrieved day month year. {*Software or code.*}

Examples follow.

Christian SL, McDonough J, Liu C-Y, Shaikh S, Vlamakis V, Badner JA, Chakravarti A, Gershon ES. 2002. Data from "An evaluation of the assembly of an approximately 15-Mb region on human chromosome 13q32-q33 linked to bipolar disorder and schizophrenia." GenBank https://www.ncbi.nlm.nih.gov/nuccore/AF339794 (accession no. AF339794). {*Accession number.*}

Sun Z. 2013. Reprocessed: in-depth membrane proteomic study of breast cancer tissues. ProteomeXchange http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=RPXD000665 (accession number requested). {*Unassigned accession number.*}

Hogle S. 2015. Supplemental material for Hogle et al. 2015 mBio. figshare https://doi.org/10.6084/m9.figshare.1533034.v1. Retrieved 16 March 2017. {*Code and/or software.*}

Nesbitt HK, Moore JW. 2016. Data from "Species and population diversity in Pacific salmon fisheries underpin indigenous food security." Dryad Digital Repository https://doi.org/10.5061/dryad.ng8pf. {*Data set in repository.*}.

Manuscript submissions that have appeared in preprint archives should cite the preprint in References, and the fact that a paper has appeared online before should be mentioned parenthetically at the end of the introductory section: (This article was submitted to an online preprint archive [1].) The reference should take the form noted above in reference 18.

(ii) References cited in the text. References that should be cited in the text include the following:

- Unpublished data
- Manuscripts submitted for publication
- Unpublished conference presentations (e.g., a report or poster that has not appeared in published conference proceedings)
- Personal communications
- Patent applications and patents pending
- Websites

These references should be made parenthetically in the text as follows:

. . . similar results (R. B. Layton and C. C. Weathers, unpublished data).
. . . system was used (J. L. McInerney, A. F. Holden, and P. N. Brighton, submitted for publication).
. . . as described previously (M. G. Gordon and F. L. Rattner, presented at the Fourth Symposium on Food Microbiology, Overton, IL, 13 to 15 June 1989). {*For nonpublished abstracts and posters, etc.*}
. . . this new process (V. R. Smoll, 20 June 1999, Australian Patent Office). {*For non-U.S. patent applications, give the date of publication of the application.*}
. . . as suggested by the World Health Organization (http://www.who.int/campaigns/immunization-week/2017/en/).

URLs for companies that produce any of the products mentioned in your study or for products being sold may not be included in the article. However, company URLs that permit access to scientific data related to the study or to shareware used in the study are permitted.

(iii) Citations in abstracts. Because the abstract must be able to stand apart from the article, references cited in it should be clear without recourse to the References section. Use an abbreviated form of citation, omitting the article title, as follows.

(P. S. Satheshkumar, A. S. Weisberg, and B. Moss, J Virol 87:10700–10709, 2013, doi:10.1128/JVI.01258-13)
(J. H. Coggin, Jr., p. 93–114, *in* D. O. Fleming and D. L. Hunt, ed., *Biological Safety. Principles and Practices*, 4th ed., 2006)
". . . in a recent report by D. A. Hopwood (mBio 4:e00612-13, 2013, doi:10.1128/mBio00612-13) . . . ."

This style should also be used for Addenda in Proof.

(iv) References related to supplemental material. If references must be cited in the supplemental material, list them in a **separate** References section within the supplemental material and cite them by those numbers; do not simply include citations of numbers from the reference list of the associated article. If the same reference(s) is to be cited in both the article itself and the supplemental material, then that reference would be listed in both References sections.

Short-Form Papers

The Short-Form format is intended for the presentation of brief observations that do not warrant full-length Research Articles. Submit Short-Form papers in the same way as Research Articles. They receive the same review, they are not published more rapidly than Research Articles, and they are not considered preliminary communications.

The title, running title (not to exceed 54 characters and spaces), byline, and correspondent footnote should be prepared as for a Research Article. Each Short-Form paper must have an abstract of no more than 75 words. Do not use section headings in the body of the paper; combine methods, results, and discussion in a single section. Paragraph lead-ins are permissible. The text should be kept to a minimum and if possible should not exceed 1,000 words; the number of figures and tables should also be kept to a minimum. Materials and methods should be described in the text, not in figure legends or table footnotes. Present acknowledgments as in Research Articles. The References section is identical to that of Research Articles.

Minireviews

Minireviews are brief (**limit of 6,000 words exclusive of references**) biographical profiles, historical perspectives, or summaries of developments in fast-moving areas of chemotherapy. They must be based on published articles; they are not outlets for unpublished data. They may address any subject within the scope of AAC. For example, subject matter may range from structure-activity correlates among a group of semisynthetic cephalosporins to the comparative efficacies of new and old drugs in the prevention or treatment of diseases of microbial origin in humans.

Minireviews may be either solicited or proffered by authors responding to a recognized need. Irrespective of origin, Minireviews are subject to review and should be submitted via the eJP online manuscript submission and peer review system. The cover letter should state whether the article was solicited and by whom.

Minireviews must have abstracts. Limit the abstract to 250 words or fewer. The body of the Minireview may have section headings and/or paragraph lead-ins.

Author bios. At the editor's invitation, corresponding authors of minireviews may submit a short biographical sketch and photo for each author for publication with the article. Biographical information should be submitted at the modification stage.

- The text limit is 150 words for each author and should include WHO you are (your name), WHERE you received your education, WHAT positions you have held and at WHICH institutions, WHERE you are now

(your current institution), WHY you have this interest, and HOW LONG you have been in this field.
- The photo should be a black-and-white head shot of passport size. Photos will be reduced to approximately 1.125 inches wide by 1.375 inches high. Photos must meet the production criteria for regular figures and should be checked for production quality by using Rapid Inspector, provided at the following URL: http://rapidinspector.cadmus.com/RapidInspector/zmw/index.jsp.
- To submit, upload the text and photos with your modified manuscript in the eJP online manuscript submission and peer review system. Include the biographical text after the References section of your manuscript, in the same file. Upload the head shots in the submission system as a "Minireview Bio Photo"; **include the author's name or enough of it for identification in each photo's file name.**

Contact the scientific editor if you have questions about what to write. Contact the production editor if you have questions about submitting your files.

Commentaries

Commentaries are invited communications concerning topics relevant to the readership of AAC and are intended to engender discussion. Reviews of the literature, methods and other how-to papers, and responses targeted at a specific published paper are not appropriate. Commentaries are subject to review.

The length may not exceed 4,000 words, and the format is like that of a Minireview (see above) except that the abstract is limited to 75 words.

Challenging Clinical Cases in Antimicrobial Resistance

Challenging Clinical Cases are brief articles designed to familiarize and provide guidance to the reader on the clinical approach to the treatment of real, challenging cases involving multidrug-resistant organisms (bacteria, viruses, fungi, and parasites). This section is focused on providing an up-to-date scientific rationale for choosing specific antimicrobials based on available clinical, microbiological, and pharmacological data and on discussing the impact of mechanisms of resistance on the outcomes for infected patients. These articles may discuss novel therapeutic strategies for treating patients infected with multidrug-resistant organisms. Only highly interesting cases that have important mechanistic and epidemiological or novel microbiological insights will be selected for review.

The article should include (i) a brief abstract (limit of 75 words); (ii) a case section describing a single clinical case up to the point when the organism is isolated, characterization of the organism, and information about susceptibility testing, when appropriate; (iii) interesting photos, figures, and/or tables (limit of two combined) highlighting the clinical presentation (see "Illustrations and Tables" below for guidelines on acceptable file types, resolution, size, etc.); (iv) a single multiple-choice question addressing the most relevant therapeutic issues (How would you interpret the susceptibility report? Which antimicrobials would be best for the patient presented in the case and why? What are the underlying mechanisms of resistance? Are there any particular pharmacological strategies, in terms of drug administration, delivery, etc., that could help in treating this patient?) with several possible answers as choices; (v) a description of the treatment strategy and patient outcome; and (vi) a reference list containing no more than 10 references. Sections ii and v above (case presentation and strategy/outcome) must not exceed 1,200 words combined.

An expert in the field (a reviewer) will discuss the case in a brief commentary section and explore answers to the questions posed by the author. (The commentator's name and role will appear at the end of the published article byline.)

These articles will be made freely available to readers at the time of publication. No page charges will be associated with these articles, but the standard fee for accepted supplemental material, if any, applies. In an attempt to stimulate conversations and engagement, readers will be able to add comments via an online feature.

Letters to the Editor

Two types of Letters to the Editor may be submitted. The first type (Comment Letter) is intended for comments on final, typeset articles published in the journal (not on accepted manuscripts posted online) and must cite published references to support the writer's argument. The second type (New-Data Letter) may report new, concise findings that are not appropriate for publication as Research Articles or Short-Form papers.

Letters may be **no more than 500 words long and must be typed double-spaced**. Refer to a recently published Letter for correct formatting. Note that authors and affiliations are listed below the title.

All Letters to the Editor must be submitted electronically, and the type of Letter (New Data or Comment) must be selected from the choices in the submission form. For Letters commenting on published articles, the cover letter should state the volume and issue in which the article was published, the title of the article, and the last name of the first author. In the Abstract section of the submission form, put "Not Applicable." Letters to the Editor do not have abstracts. Both types of Letter must have a title, which must appear on the manuscript and on the submission form. Figures and tables should be kept to a minimum.

If the Letter is related to a published article, it will be sent to the editor who handled the article in question. If the editor believes that publication is warranted, he/she will solicit a reply from the corresponding author of the article and give approval for publication.

New-Data Letters will be assigned to an editor according to subject matter and will be reviewed by that editor and/or a reviewer.

Please note that some indexing/abstracting services do not include Letters to the Editor in their databases.

Errata

Errata provide a means of correcting errors that occurred during the writing, typing, editing, or publication (e.g., a

misspelling, a dropped word or line, or mislabeling in a figure) of a published article. Submit Errata via the eJP online manuscript submission and peer review system (see "Submission, Review, and Publication Processes"). In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Erratum as a Microsoft Word file. Please see a recent issue for correct formatting.

Author Corrections

Author Corrections provide a means of correcting errors of omission (e.g., author names or citations) and errors of a scientific nature that do not alter the overall basic results or conclusions of a published article (e.g., an incorrect unit of measurement or order of magnitude used throughout, contamination of one of numerous cultures, or misidentification of a mutant strain, causing erroneous data for only a [noncritical] portion of the study). Note that the addition of new data is not permitted.

For corrections of a scientific nature or issues involving authorship, including contributions and use or ownership of data and/or materials, all disputing parties must agree, in writing, to publication of the Correction. For omission of an author's name, letters must be signed by the authors of the article and the author whose name was omitted. The editor who handled the article will be consulted if necessary.

Submit an Author Correction via the eJP online manuscript submission and peer review system (see "Submission, Review, and Publication Processes"). Select Author Correction as the manuscript type. In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Author Correction as a Microsoft Word file. Please see a recent issue for correct formatting. Signed letters of agreement must be supplied as supplemental material not for publication (scanned PDF files).

Retractions

Retractions are reserved for major errors or breaches of ethics that, for example, may call into question the source of the data or the validity of the results and conclusions of an article. Submit Retractions via the eJP online manuscript submission and peer review system (see "Submission, Review, and Publication Processes"). In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Retraction as a Microsoft Word file. Letters of agreement signed by all of the authors must be supplied as supplemental material not for publication (scanned PDF files). The Retraction will be assigned to the editor in chief of the journal, and the editor who handled the paper and the chairperson of the ASM Journals Board will be consulted. If all parties agree to the publication and content of the Retraction, it will be sent to the Journals Department for publication.

CrossMark

ASM has implemented CrossMark. CrossMark is a multipublisher initiative to provide a standard way for readers to locate the current version of an article. Clicking on the CrossMark logo will indicate whether an article is current or whether updates have been published. Additional information about CrossMark can be found on CrossMark's website and on ASM's CrossMark policy page.

ILLUSTRATIONS AND TABLES

Illustrations

Image manipulation. Digital images submitted for publication may be inspected by ASM production specialists for any manipulations or electronic enhancements that may be considered to be the result of scientific misconduct based on the guidelines provided below. Any images/data found to contain manipulations of concern will be referred to the editor in chief, and authors may then be requested to provide their primary data for comparison with the submitted image file. Investigation of the concerns may delay publication and may result in revocation of acceptance and/or additional action by ASM.

Linear adjustments to contrast, brightness, and/or color are generally acceptable, as long as the measures taken are necessary to view elements that are already present in the data and the adjustments are applied to the entire image and not just specific areas. Unacceptable adjustments to images include, but are not limited to, the removal or deletion, concealment, duplication (copying and pasting), addition, selective enhancement, or repositioning of elements within the image.

Nonlinear adjustments made to images, such as changes to gamma settings, should be fully disclosed in the figure legends at the time of submission. In addition, images created by compiling multiple files, including noncontiguous portions of the same image, should clearly convey that these multiple files are not a single image. This can be done by "tooling," or inserting thin lines, between the individual images.

File types and formats. Illustrations may be continuous-tone images, line drawings, or composites. Color graphics may be submitted. Suggestions about how to ensure accurate color reproduction are given below.

On initial submission, figures may be uploaded as individual PDF files or combined and uploaded as a single PDF file. Place each legend in the text file, as well as on the same page with the corresponding figure to assist review. At the modification stage, production-quality digital files must be provided. Because the legends will be copyedited and typeset for final publication, they should appear within the main text, after the References section, and should not be included as part of the figure itself at this stage. All graphics submitted with modified manuscripts must be bitmap, grayscale, or in the RGB (preferred) or CMYK color mode. See "Color illustrations." Halftone images (those with various densities or shades) must be grayscale, not bitmap. AAC accepts TIFF or EPS files but discourages PowerPoint for either black-and-white or color images.

For instructions on creating acceptable EPS and TIFF files, refer to the Cadmus digital art website, http://art.cadmus.com/da/index.jsp. PowerPoint requires users to pay close attention to the fonts used in their images (see the section on fonts below). If

instructions for fonts are not followed exactly, images prepared for publication are subject to missing characters, improperly converted characters, or shifting/obscuring of elements or text in the figure. For proper font use in PowerPoint images, refer to the Cadmus digital art website, http://art.cadmus.com/da/instructions/ppt_disclaimer.jsp. Note that, due to page composition system requirements, you must verify that your PowerPoint files can be converted to PDF without any errors.

We strongly recommend that before returning their modified manuscripts, authors check the acceptability of their digital images for production by running their files through Rapid Inspector, a tool provided at the following URL: http://rapidinspector.cadmus.com/RapidInspector/zmw/index.jsp. Rapid Inspector is an easy-to-use, Web-based application that identifies file characteristics that may render the image unusable for production. Please note when using Rapid Inspector to check PowerPoint files that there is a known bug in the application that can occasionally fail PowerPoint Presentation (.pptx) files, even though the files meet all required production criteria. If you experience this bug, the issue can be corrected by saving the PowerPoint files as an older version, PowerPoint 97-2004 Presentation (.ppt), during the Save As process (use the drop-down format menu and select this format). Once you save your files as .ppt, they will pass Rapid Inspector if all required production criteria have been met.

If you have additional questions about using the Rapid Inspector preflighting tool, please send an e-mail inquiry to helpdesk.digitalartsupport@cenveo.com.

Minimum resolution. It is extremely important that a high enough file resolution is used. All separate images that you import into a figure file must be at the correct resolution before they are placed. (For instance, placing a 72-dpi image in a 300-dpi EPS file will not result in the placed image meeting the minimum requirements for file resolution.) Note, however, that the higher the resolution, the larger the file and the longer the upload time. Publication quality will not be improved by using a resolution higher than the minimum. Minimum resolutions are as follows:

- 300 dpi for grayscale and color
- 600 dpi for combination art (lettering and images)
- 1,200 dpi for line art

Size. All graphics **should be submitted at their intended publication size** so that no reduction or enlargement is necessary. Resolution must be at the required level at the submitted size. Include only the significant portion of an illustration. White space must be cropped from the image, and excess space between panel labels and the image must be eliminated.

- Maximum figure width: 6.875 inches (ca. 17.4 cm)
- Maximum figure height: 9.0625 inches (23.0 cm)

Contrast. Illustrations must contain sufficient contrast to be viewed easily on a monitor or on the printed page.

Labeling and assembly. All final lettering and labeling must be incorporated into the figures. On initial submission, illustrations should be provided as PDF files, with the legends in the text file and with a legend beneath each image to assist review. At the modification stage, production-quality digital figure files (without legends) must be provided. Put the figure number well outside the boundaries of the image itself. (Numbering may need to be changed at the copyediting stage.) Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file; i.e., rather than uploading a separate file for each panel in a figure, assemble all panels in one piece and supply them as one file.

Fonts. To avoid font problems, set all type in one of the following fonts: Arial, Helvetica, Times Roman, European PI, Mathematical PI, or Symbol. Courier may be used but should be limited to nucleotide or amino acid sequences in which a nonproportional (monospace) font is required. All fonts other than these must be converted to paths (or outlines) in the application with which they were created.

Color illustrations. All figures submitted in color will be processed as color. Adherence to the following guidelines will help to ensure color reproduction that is as accurate as possible.

The final online version is considered the version of record for AAC and all other ASM journals. To maximize online reproduction, color illustrations should be supplied in the RGB color mode as either (i) RGB TIFF images with a resolution of at least 300 pixels per inch (raster files, consisting of pixels) or (ii) Illustrator-compatible EPS files with RGB color elements (vector files, consisting of lines, fonts, fills, and images). CMYK files are also accepted. Other than in color space, CMYK files must meet the same production criteria as RGB files. The RGB color space is the native color space of computer monitors and of most of the equipment and software used to capture scientific data, and it can display a wider range of colors (especially bright fluorescent hues) than the CMYK (cyan, magenta, yellow, black) color space used by print devices that put ink (or toner) on paper. For reprints, ASM's print provider will automatically create CMYK versions of color illustrations from the supplied RGB versions. Color in the reprints may not match that in the online journal of record because of the smaller range of colors capable of being reproduced by CMYK inks on a printing press. For additional information on RGB versus CMYK color, refer to the Cadmus digital art site, http://art.cadmus.com/da/guidelines_rgb.jsp.

Drawings. Submit graphs, charts, complicated chemical or mathematical formulas, diagrams, and other drawings as finished products not requiring additional artwork or typesetting. All elements, including letters, numbers, and symbols, must be easily readable, and both axes of a graph must be labeled.

When creating line art, please use the following guidelines:

(i) **All art must be submitted at its intended publication size.** For acceptable dimensions, see "Size," above.

(ii) **Avoid using screens (i.e., shading) in line art.** It can be difficult and time-consuming to reproduce these images without moiré patterns. Various pattern backgrounds are preferable to screens as long as the patterns are not imported from another application. If you must use images containing screens,

(a) Generate the image at line screens of 85 lines per inch or less.

(b) When applying multiple shades of gray, differentiate the gray levels by at least 20%.

(c) Never use levels of gray below 5% or above 95% as they are likely to fade out or become totally black when output.

(iii) Use thick, solid lines that are no finer than 1 point in thickness.

(iv) Use type that is no smaller than 6 points at the final publication size.

(v) Avoid layering type directly over shaded or textured areas.

(vi) Avoid the use of reversed type (white lettering on a black background).

(vii) Avoid heavy letters, which tend to close up, and unusual symbols, which the printer may not be able to reproduce in the legend.

(viii) If colors are used, avoid using similar shades of the same color and avoid very light colors.

In figure ordinate and abscissa scales (as well as table column headings), avoid the ambiguous use of numbers with exponents. Usually, it is preferable to use the Système International d'Unités (SI) symbols (μ for 10^{-6}, m for 10^{-3}, k for 10^{3}, and M for 10^{6}, etc.). Thus, a representation of 20,000 cpm on a figure ordinate should be made by the number 20 accompanied by the label kcpm. A complete listing of SI symbols can be found in the International Union of Pure and Applied Chemistry (IUPAC) publication *Quantities, Units and Symbols in Physical Chemistry*, 3rd ed. (RSC Publishing, Cambridge, United Kingdom, 2007), and at https://www.nist.gov/physical-measurement-laboratory/special-publication-811.

When powers of 10 must be used, the journal requires that the exponent power be associated with the number shown. In representing 20,000 cells per ml, the numeral on the ordinate should be "2" and the label should be "10^{4} cells per ml" (not "cells per ml $\times$ 10^{-4}"). Likewise, an enzyme activity of 0.06 U/ml might be shown as 6 accompanied by the label "10^{-2} U/ml." The preferred designation is 60 mU/ml (milliunits per milliliter).

Presentation of Nucleic Acid Sequences

Long nucleic acid sequences must be presented as figures in the following format to conserve space. Print the sequence in lines of approximately 100 to 120 nucleotides in a nonproportional (monospace) font that is easily legible when published with a line length of 6 inches (ca. 15.2 cm). If possible, lines of nucleic acid sequence should be further subdivided into blocks of 10 or 20 nucleotides by spaces within the sequence or by marks above it. Uppercase and lowercase letters may be used to designate the exon-intron structure or transcribed regions, etc., if the lowercase letters remain legible at a 6-inch (ca. 15.2-cm) line length. Number the sequence line by line; place numerals representing the first base of each line to the left of the lines. Minimize spacing between lines of sequence, leaving room only for annotation of the sequence. Annotation may include boldface, underlining, brackets, and boxes, etc. Encoded amino acid sequences may be presented, if necessary, immediately above or below the first nucleotide of each codon, by using the single-letter amino acid symbols. Comparisons of multiple nucleic acid sequences should conform as nearly as possible to the same format.

Figure Legends

On initial submission, each legend should be placed in the text file *and* be incorporated into the image file beneath the figure to assist review.

Legends should provide enough information so that the figure is understandable without frequent reference to the text. However, detailed experimental methods must be described in the Materials and Methods section, not in a figure legend. A method that is unique to one of several experiments may be set forth in a legend only if the description is very brief (one or two sentences). Define all symbols used in the figure and define all abbreviations that are not used in the text.

Tables

Tables that contain artwork, chemical structures, or complex shading must be submitted as illustrations in an acceptable format at the modification stage. The preferred format for regular tables is Microsoft Word; however, WordPerfect and Acrobat PDF are also acceptable. Note that a straight Excel file is not currently an acceptable format. Excel files must be either embedded in a Word or WordPerfect document or converted to PDF before being uploaded.

Tables should be formatted as follows. Arrange the data so that **columns of like material read down, not across.** The headings should be sufficiently clear so that the meaning of the data is understandable without reference to the text. See the "Abbreviations" section of these Instructions for those that should be used in tables. Explanatory footnotes are acceptable, but more-extensive table "legends" are not. Footnotes should not include detailed descriptions of the experiment. Tables must include enough information to warrant table format; those with fewer than six pieces of data will be incorporated into the text by the copy editor. Table 1 is an example of a well-constructed table.

Avoid tables (or figures) of raw data on drug susceptibility, therapeutic activity, or toxicity. Such data should be analyzed by an approved procedure, and the results should be presented in tabular form.

TABLE 1 Distribution of protein and ATPase in fractions of dialyzed membranes[a]

Membrane	Fraction	ATPase	
		U/mg of protein	Total U
Control	Depleted membrane	0.036	2.3
	Concentrated supernatant	0.134	4.82
E1 treated	Depleted membrane	0.034	1.98
	Concentrated supernatant	0.11	4.6

[a] Specific activities of ATPase of nondepleted membranes from control and treated bacteria were 0.21 and 0.20, respectively.

NOMENCLATURE

Chemical and Biochemical Nomenclature

The recognized authority for the names of chemical compounds is *Chemical Abstracts* (CAS; http://www.cas.org/) and its indexes. *The Merck Index Online* (https://www.rsc.org/merck-index) is also an excellent source. For guidelines to the use of biochemical terminology, consult *Biochemical Nomenclature and Related Documents* (Portland Press, London, United Kingdom, 1992), available at http://www.sbcs.qmul.ac.uk/iupac/bibliog/white.html, and the Instructions to Authors of the *Journal of Biological Chemistry*.

Molecular weight should not be expressed in daltons; molecular weight is a unitless ratio. Molecular mass is expressed in daltons.

For enzymes, use the recommended (trivial) name as assigned by the Nomenclature Committee of the International Union of Biochemistry (IUB) as described in *Enzyme Nomenclature* (Academic Press, Inc., New York, NY, 1992) and its supplements and at http://www.sbcs.qmul.ac.uk/iubmb/enzyme/. If a nonrecommended name is used, place the proper (trivial) name in parentheses at first use in the abstract and text. Use the EC number when one has been assigned. Authors of papers describing enzymological studies should review the standards of the STRENDA Commission for information required for adequate description of experimental conditions and for reporting enzyme activity data (http://www.beilstein-institut.de/en/projects/strenda/guidelines).

Nomenclature of Microorganisms

Binary names, consisting of a generic name and a specific epithet (e.g., *Escherichia coli*), must be used for all microorganisms. Names of categories at or above the genus level may be used alone, but specific and subspecific epithets may not. A specific epithet must be preceded by a generic name, written out in full the first time it is used in a paper. Thereafter, the generic name should be abbreviated to the initial capital letter (e.g., *E. coli*), provided there can be no confusion with other genera used in the paper. Names of all bacterial taxa (kingdoms, phyla, classes, orders, families, genera, species, and subspecies) are printed in italics and should be italicized in the manuscript; strain designations and numbers are not. Vernacular (common) names should be in lowercase roman type (e.g., streptococcus, brucella). For *Salmonella*, genus, species, and subspecies names should be rendered in standard form: *Salmonella enterica* at first use, *S. enterica* thereafter; *Salmonella enterica* subsp. *arizonae* at first use, *S. enterica* subsp. *arizonae* thereafter. Names of serovars should be in roman type with the first letter capitalized: *Salmonella enterica* serovar Typhimurium. After the first use, the serovar may also be given without a species name: *Salmonella* Typhimurium, *S.* Typhimurium, or *Salmonella* serovar Typhimurium. For other information regarding serovar designations, see *Antigenic Formulae of the Salmonella Serovars*, 9th ed. (P. A. D. Grimont and F.-X. Weill, WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France, 2007; see http://www.scacm.org/free/Antigenic%20Formulae%20of%20the%20Salmonella%20Serovars%202007%209th%20edition.pdf). For a summary of the current standards for *Salmonella* nomenclature and the Kaufmann-White criteria, see the article by Brenner et al. (J Clin Microbiol 38:2465–2467, 2000), the opinion of the Judicial Commission of the International Committee on Systematics of Prokaryotes (Int J Syst Evol Microbiol 55:519–520, 2005), and the article by Tindall et al. (Int J Syst Evol Microbiol 55:521–524, 2005).

The spelling of bacterial names should follow the *Approved Lists of Bacterial Names (Amended) & Index of the Bacterial and Yeast Nomenclatural Changes* (V. B. D. Skerman et al., ed., American Society for Microbiology, Washington, DC, 1989) and the validation lists and notification lists published in the *International Journal of Systematic and Evolutionary Microbiology* (formerly the *International Journal of Systematic Bacteriology*) since January 1989. In addition, two sites on the World Wide Web list current approved bacterial names: Prokaryotic Nomenclature Up-to-Date (https://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date.html) and List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.net/). If there is reason to use a name that does not have standing in nomenclature, the name should be enclosed in quotation marks in the title and at its first use in the abstract and the text and an appropriate statement concerning the nomenclatural status of the name should be made in the text. "*Candidatus*" species should always be set in quotation marks.

Since the classification of fungi is not complete, it is the responsibility of the author to determine the accepted binomial for a given organism. Sources for these names include *The Yeasts: a Taxonomic Study*, 5th ed. (C. P. Kurtzman, J. W. Fell, and T. Boekhout, ed., Elsevier Science, Amsterdam, Netherlands, 2011), and *Dictionary of the Fungi*, 10th ed. (P. M. Kirk, P. F. Cannon, D. W. Minter, and J. A. Stalpers, ed., CABI International, Wallingford, Oxfordshire, United Kingdom, 2008); see also http://www.speciesfungorum.org/Names/Fundic.asp.

Names used for viruses should be those approved by the International Committee on Taxonomy of Viruses (ICTV) and reported on the ICTV Virus Taxonomy website (https://talk.ictvonline.org/). In addition, the recommendations of the ICTV regarding the use of species names should generally be followed: when the entire species is discussed as a taxonomic entity, the species name, as with other taxa, is italic and has the first letter and any proper nouns capitalized (e.g., *Tobacco mosaic virus*, *Murray Valley encephalitis virus*). When the behavior or manipulation of individual viruses is discussed, the vernacular (e.g., tobacco mosaic virus, Murray Valley encephalitis virus) should be used. If desired, synonyms may be added parenthetically when the

name is first mentioned. Approved generic (or group) and family names may also be used.

Microorganisms, viruses, and plasmids should be given designations consisting of letters and serial numbers. It is generally advisable to include a worker's initials or a descriptive symbol of locale or laboratory, etc., in the designation. Each new strain, mutant, isolate, or derivative should be given a new (serial) designation. This designation should be distinct from those of the genotype and phenotype, and genotypic and phenotypic symbols should not be included. Plasmids are named with a lowercase "p" followed by the designation in uppercase letters and numbers. To avoid the use of the same designation as that of a widely used strain or plasmid, check the designation against a publication database such as Medline.

Genetic Nomenclature

To facilitate accurate communication, **it is important that standard genetic nomenclature be used whenever possible and that deviations or proposals for new naming systems be endorsed by an appropriate authoritative body**. Review and/or publication of submitted manuscripts that contain new or nonstandard nomenclature may be delayed by the editor or the Journals Department so that they may be reviewed.

Bacteria. The genetic properties of bacteria are described in terms of phenotypes and genotypes. The phenotype describes the observable properties of an organism. The genotype refers to the genetic constitution of an organism, usually in reference to some standard wild type. The guidelines that follow are based on the recommendations of Demerec et al. (Genetics 54:61–76, 1966).

(i) Phenotype designations must be used when mutant loci have not been identified or mapped. They can also be used to identify the protein product of a gene, e.g., the OmpA protein. Phenotype designations generally consist of three-letter symbols; these are not italicized, and the first letter of the symbol is capitalized. It is preferable to use Roman or Arabic numerals (instead of letters) to identify a series of related phenotypes. Thus, a series of nucleic acid polymerase mutants might be designated Pol1, Pol2, and Pol3, etc. Wild-type characteristics can be designated with a superscript plus (Pol^+), and, when necessary for clarity, negative superscripts (Pol^-) can be used to designate mutant characteristics. Lowercase superscript letters may be used to further delineate phenotypes (e.g., Str^r for streptomycin resistance). Phenotype designations should be defined.

(ii) Genotype designations are also indicated by three-letter locus symbols. In contrast to phenotype designations, these are lowercase italic (e.g., *ara his rps*). If several loci govern related functions, these are distinguished by italicized capital letters following the locus symbol (e.g., *araA araB araC*). Promoter, terminator, and operator sites should be indicated as described by Bachmann and Low (Microbiol Rev 44:1–56, 1980): e.g., *lacZp*, *lacAt*, and *lacZo*.

(iii) Wild-type alleles are indicated with a superscript plus (ara^+ his^+). A superscript minus is not used to indicate a mutant locus; thus, one refers to an *ara* mutant rather than an ara^- strain.

(iv) Mutation sites are designated by placing serial isolation numbers (allele numbers) after the locus symbol (e.g., *araA1* *araA2*). If only a single such locus exists or if it is not known in which of several related loci the mutation has occurred, a hyphen is used instead of the capital letter (e.g., *ara-23*). It is essential in papers reporting the isolation of new mutants that allele numbers be given to the mutations. For *Escherichia coli*, there is a registry of such numbers: *E. coli* Genetic Stock Center (http://cgsc2.biology.yale.edu/). For the genus *Salmonella*, the registry is *Salmonella* Genetic Stock Center (http://people.ucalgary.ca/~kesander/). For the genus *Bacillus*, the registry is *Bacillus* Genetic Stock Center (http://www.bgsc.org/).

(v) The use of superscripts with genotypes (other than + to indicate wild-type alleles) should be avoided. Designations indicating amber mutations (Am), temperature-sensitive mutations (Ts), constitutive mutations (Con), cold-sensitive mutations (Cs), production of a hybrid protein (Hyb), and other important phenotypic properties should follow the allele number [e.g., *araA230*(Am) *hisD21*(Ts)]. All other such designations of phenotype must be defined at the first occurrence. If superscripts must be used, they must be approved by the editor and defined at the first occurrence in the text.

Subscripts may be used in two situations. Subscripts may be used to distinguish between genes (having the same name) from different organisms or strains; e.g., $his_{E.\ coli}$ or $his_{K\text{-}12}$ for the *his* gene of *E. coli* or strain K-12, respectively, may be used to distinguish this gene from the *his* gene in another species or strain. An abbreviation may also be used if it is explained. Similarly, a subscript is also used to distinguish between genetic elements that have the same name. For example, the promoters of the *gln* operon can be designated $glnAp_1$ and $glnAp_2$. This form departs slightly from that recommended by Bachmann and Low (e.g., *desC1p*).

(vi) Deletions are indicated by the symbol Δ placed before the deleted gene or region, e.g., Δ*trpA432*, Δ(*aroP-aceE*)*419*, or Δ(*hisQ-hisJo*)*1256*. Similarly, other symbols can be used (with appropriate definition). Thus, a fusion of the *ara* and *lac* operons can be shown as Φ(*ara-lac*)*95*. Likewise, Φ(*araB′-lacZ*$^+$)*96* indicates that the fusion results in a truncated *araB* gene fused to an intact *lacZ* gene, and Φ(*malE-lacZ*)*97*(Hyb) shows that a hybrid protein is synthesized. An inversion is shown as IN(*rrnD-rrnE*)*1*. An insertion of an *E. coli his* gene into plasmid pSC101 at zero kilobases (0 kb) is shown as pSC101 Ω(0kb::K-12*hisB*)*4*. An alternative designation of an insertion can be used in simple cases, e.g., *galT236*::Tn*5*. The number *236* refers to the locus of the insertion, and if the strain carries an additional *gal* mutation, it is listed separately. Additional examples, which utilize a slightly different format, can be found in the papers by Campbell et al. and Novick et al. cited below. It is important in reporting the construction of strains in which a mobile element was inserted and subsequently deleted that this fact be noted in the strain table. This can be done by listing the genotype of the strain used as an intermediate in a table footnote or by making a direct or parenthetical remark in the genotype, e.g., (F^-), ΔMu *c*ts, or *mal*::ΔMu *c*ts::*lac*. In setting parenthetical remarks within the genotype or dividing the genotype into constituent elements, parentheses and brackets are used without special meaning; brackets are used outside parentheses. To indicate the presence of an episome, parentheses (or brackets) are used (λ, F^+). Reference to an integrated episome is

indicated as described above for inserted elements, and an exogenote is shown as, for example, W3110/F′8(*gal*$^+$).

For information about genetic maps of locus symbols in current use, consult Berlyn (Microbiol Mol Biol Rev 62:814–984, 1998) for *E. coli* K-12, Sanderson and Roth (Microbiol Rev 52:485–532, 1988) for *Salmonella* serovar Typhimurium, Holloway et al. (Microbiol Rev 43:73–102, 1979) for the genus *Pseudomonas*, Piggot and Hoch (Microbiol Rev 49:158–179, 1985) for *Bacillus subtilis*, Perkins et al. (Microbiol Rev 46:426–570, 1982) for *Neurospora crassa*, and Mortimer and Schild (Microbiol Rev 49:181–213, 1985) for *Saccharomyces cerevisiae*. For yeasts, *Chlamydomonas* spp., and several fungal species, symbols such as those given in the *Handbook of Microbiology*, 2nd ed. (A. I. Laskin and H. A. Lechevalier, ed., CRC Press, Inc., Cleveland, OH, 1988) should be used.

Conventions for naming genes. It is recommended that (entirely) new genes be given names that are mnemonics of their function, avoiding names that are already assigned and earlier or alternative gene names, irrespective of the bacterium for which such assignments have been made. Similarly, it is recommended that, whenever possible, orthologous genes present in different organisms receive the same name. When homology is not apparent or the function of a new gene has not been established, a provisional name may be given by one of the following methods. (i) The gene may be named on the basis of its map location in the style *yaaA*, analogous to the style used for recording transposon insertions (*zef*) as discussed below. A list of such names in use for *E. coli* has been published by Rudd (Microbiol Mol Biol Rev 62:985–1019, 1998). (ii) A provisional name may be given in the style described by Demerec et al. (e.g., *usg*, gene upstream of *folC*). Such names should be unique, and names such as *orf* or *genX* should not be used. For reference, the *E. coli* Genetic Stock Center's database includes an updated listing of *E. coli* gene names and gene products. It is accessible on the Internet (http://cgsc2.biology.yale.edu/index.php). A list can also be found in the work of Riley (Microbiol Rev 57:862–952, 1993). For the genes of other bacteria, consult the references given above.

For prokaryotes, gene names should not begin with prefixes indicating the genus and species from which the gene is derived. (However, subscripts may be used where necessary to distinguish between genes from different organisms or strains as described in section v of "Bacteria," above.) For eukaryotes, such prefixes may be used for clarity when discussing genes with the same name from two different organisms (e.g., Sc*URA3* versus Ca*URA3*); the prefixes are not considered part of the gene name proper and are not italicized.

Locus tags. Locus tags are systematic, unique identifiers that are assigned to each gene in GenBank. All genes mentioned in a manuscript should be traceable to their sequences by the reader, and locus tags may be used for this purpose in manuscripts to identify uncharacterized genes. In addition, authors should check GenBank to make sure that they are using the correct, up-to-date format for locus tags (e.g., uppercase versus lowercase letters and the presence or absence of an underscore, etc.). Locus tag formats vary between different organisms and also may be updated for a given organism, so it is important to check GenBank at the time of manuscript preparation.

"Mutant" versus "mutation." Keep in mind the distinction between a mutation (an alteration of the primary sequence of the genetic material) and a mutant (a strain carrying one or more mutations). One may speak about the mapping of a mutation, but one cannot map a mutant. Likewise, a mutant has no genetic locus, only a phenotype.

"Homology" versus "similarity." For use of terms that describe relationships between genes, consult the articles by Theissen (Nature 415:741, 2002) and Fitch (Trends Genet 16:227–231, 2000). "Homology" implies a relationship between genes that have a common evolutionary origin; partial homology is not recognized. When sequence comparisons are discussed, it is more appropriate to use the term "percent sequence similarity" or "percent sequence identity," as appropriate.

Strain designations. Do not use a genotype as a name (e.g., ". . . subsequent use of *leuC6* for transduction . . ."). If a strain designation has not been chosen, select an appropriate word combination (e.g., "another strain containing the *leuC6* mutation").

Viruses. The genetic nomenclature for viruses differs from that for bacteria. In most instances, viruses have no phenotype, since they have no metabolism outside host cells. Therefore, distinctions between phenotype and genotype cannot be made. Superscripts are used to indicate hybrid genomes. Genetic symbols may be one, two, or three letters. For example, a mutant strain of λ might be designated λ *A*am11 *int*2 *red*114 *c*I857; this strain carries mutations in genes *c*I, *int*, and *red* and an amber-suppressible (Am) mutation in gene *A*. A strain designated λ att^{434} imm^{21} would represent a hybrid of phage λ that carries the immunity region (*imm*) of phage 21 and the attachment (*att*) region of phage 434. Host DNA insertions into viruses should be delineated by square brackets, and the genetic symbols and designations for such inserted DNA should conform to those used for the host genome. Genetic symbols for phage λ can be found in reports by Szybalski and Szybalski (Gene 7:217–270, 1979) and Echols and Murialdo (Microbiol Rev 42:577–591, 1978).

Eukaryotes. FlyBase (http://flybase.org/) is the genetic nomenclature authority for *Drosophila melanogaster*. WormBase (http://www.wormbase.org/#01-23-6) is the genetic nomenclature authority for *Caenorhabditis elegans*. When naming genes for *Aspergillus* species, the nomenclature guidelines posted at http://www.aspergillusgenome.org/Nomenclature.shtml should be followed, and the *Aspergillus* Genome Database (http://www.aspgd.org/) should be searched to ensure that any new name is not already in use. The *Saccharomyces* Genome Database (https://www.yeastgenome.org/) and the *Candida* Genome Database (http://www.candidagenome.org/) are authorities for *Saccharomyces cerevisiae* and *Candida albicans* genetic nomenclature, respectively. For information about the genetic nomenclature of other eukaryotes, see the Instructions to Authors for *Molecular and Cellular Biology*.

Transposable elements, plasmids, and restriction enzymes. Nomenclature of transposable elements (insertion sequences, transposons, and phage Mu, etc.) should follow the

recommendations of Campbell et al. (Gene 5:197–206, 1979), with the modifications given in section vi of "Bacteria," above. The Internet site where insertion sequences of eubacteria and archaea are described and new sequences can be recorded is https://www-is.biotoul.fr.

The system of designating transposon insertions at sites where there are no known loci, e.g., *zef-123*::Tn*5*, has been described by Chumley et al. (Genetics 91:639–655, 1979). The nomenclature recommendations of Novick et al. (Bacteriol Rev 40:168–189, 1976) for plasmids and plasmid-specified activities, of Low (Bacteriol Rev 36:587–607, 1972) for F′ factors, and of Roberts et al. (Nucleic Acids Res 31:1805–1812, 2003) for restriction enzymes, DNA methyltransferases, homing endonucleases, and their genes should be used whenever possible. The nomenclature for recombinant DNA molecules, constructed *in vitro*, follows the nomenclature for insertions in general. DNA inserted into recombinant DNA molecules should be described by using the gene symbols and conventions for the organism from which the DNA was obtained.

Tetracycline resistance determinants. The nomenclature for tetracycline resistance determinants is based on the proposal of Levy et al. (Antimicrob Agents Chemother 43:1523–1524, 1999). The style for such determinants is, e.g., Tet B; the space helps distinguish the determinant designation from that for phenotypes and proteins (TetB). The above-referenced article also gives the correct format for genes, proteins, and determinants in this family.

ABBREVIATIONS AND CONVENTIONS

Verb Tense

ASM strongly recommends that for clarity you use the **past** tense to narrate particular events in the past, including the procedures, observations, and data of the study that you are reporting. Use the present tense for your own general conclusions, the conclusions of previous researchers, and generally accepted facts. Thus, most of the abstract, Materials and Methods, and Results will be in the past tense, and most of the introduction and some of the Discussion will be in the present tense.

Be aware that it may be necessary to vary the tense in a single sentence. For example, it is correct to say "White (30) demonstrat*ed* that XYZ cells *grow* at pH 6.8," "Figure 2 show*s* that ABC cells fail*ed* to grow at room temperature," and "Air *was* removed from the chamber and the mice *died*, which *proves* that mice *require* air." In reporting statistics and calculations, it is correct to say "The values for the ABC cells *are* statistically significant, indicating that the drug inhibit*ed* . . . ."

For an in-depth discussion of tense in scientific writing, see *How To Write and Publish a Scientific Paper*, 7th ed.

Abbreviations

General. Abbreviations should be used as an aid to the reader, rather than as a convenience to the author, and therefore their **use should be limited.** Abbreviations other than those recommended by the IUPAC-IUB (*Biochemical Nomenclature and Related Documents*, 1992) should be used only when a case can be made for necessity, such as in tables and figures.

It is often possible to use pronouns or to paraphrase a long word after its first use (e.g., "the drug" or "the substrate"). Standard chemical symbols and trivial names or their symbols (folate, Ala, and Leu, etc.) may also be used.

Define each abbreviation and introduce it in parentheses the first time it is used; e.g., "cultures were grown in Eagle minimal essential medium (MEM)." Generally, eliminate abbreviations that are not used at least three times in the text (including tables and figure legends).

Not requiring introduction. In addition to abbreviations for Système International d'Unités (SI) units of measurement, other common units (e.g., bp, kb, and Da), and chemical symbols for the elements, the following should be used without definition in the title, abstract, text, figure legends, and tables:

DNA (deoxyribonucleic acid)
cDNA (complementary DNA)
RNA (ribonucleic acid)
cRNA (complementary RNA)
RNase (ribonuclease)
DNase (deoxyribonuclease)
rRNA (ribosomal RNA)
mRNA (messenger RNA)
tRNA (transfer RNA)
AMP, ADP, ATP, dAMP, ddATP, and GTP, etc. (for the respective 5′ phosphates of adenosine and other nucleosides) (add 2′-, 3′-, or 5′- when needed for contrast)
ATPase and dGTPase, etc. (adenosine triphosphatase and deoxyguanosine triphosphatase, etc.)
NAD (nicotinamide adenine dinucleotide)
NAD^+ (nicotinamide adenine dinucleotide, oxidized)
NADH (nicotinamide adenine dinucleotide, reduced)
NADP (nicotinamide adenine dinucleotide phosphate)
NADPH (nicotinamide adenine dinucleotide phosphate, reduced)
$NADP^+$ (nicotinamide adenine dinucleotide phosphate, oxidized)
poly(A) and poly(dT), etc. (polyadenylic acid and polydeoxythymidylic acid, etc.)
oligo(dT), etc. (oligodeoxythymidylic acid, etc.)
UV (ultraviolet)
PFU (plaque-forming units)
CFU (colony-forming units)
MIC (minimal inhibitory concentration)
Tris (tris[hydroxymethyl] aminomethane)
DEAE (diethylaminoethyl)
EDTA (ethylenediaminetetraacetic acid)
EGTA (ethylene glycol-bis[β-aminoethyl ether]-*N*,*N*,*N*′,*N*′-tetraacetic acid)
HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)
PCR (polymerase chain reaction)
AIDS (acquired immunodeficiency syndrome)

Abbreviations for cell lines (e.g., HeLa) also need not be defined.

The following abbreviations should be used without definition in tables:

amt (amount)
approx (approximately)
avg (average)
concn (concentration)
diam (diameter)
expt (experiment)
exptl (experimental)
ht (height)
mo (month)
mol wt (molecular weight)
no. (number)
prepn (preparation)
SD (standard deviation)
SE (standard error)
SEM (standard error of the mean)
sp act (specific activity)
sp gr (specific gravity)
temp (temperature)
vol (volume)
vs (versus)
wk (week)
wt (weight)
yr (year)

Drugs and pharmaceutical agents. Should an author decide to abbreviate the names of antimicrobial agents in a man-

uscript, the following standard abbreviations are strongly recommended.

(i) Antibacterial agents. Use the indicated abbreviations for the following antibacterial agents.

amikacin (AMK)
amoxicillin (AMX)
amoxicillin-clavulanic acid (AMC)
ampicillin (AMP)
ampicillin-sulbactam (SAM)
azithromycin (AZM)
azlocillin (AZL)
aztreonam (ATM)
carbenicillin (CAR)
cefaclor (CEC)
cefadroxil (CFR)
cefamandole (FAM)
cefazolin (CFZ)
cefdinir (CDR)
cefditoren (CDN)
cefepime (FEP)
cefetamet (FET)
cefixime (CFM)
cefmetazole (CMZ)
cefonicid (CID)
cefoperazone (CFP)
cefotaxime (CTX)
cefotetan (CTT)
cefoxitin (FOX)
cefpodoxime (CPD)
cefprozil (CPR)
ceftaroline (CPT)
ceftazidime (CAZ)
ceftazidime-avibactam (CZA)
ceftibuten (CTB)
ceftizoxime (ZOX)
ceftolozane-tazobactam (C/T)
ceftriaxone (CRO)
cefuroxime (axetil or sodium) (CXM)
cephalexin (LEX)
cephalothin (CEF)
cephapirin (HAP)
cephradine (RAD)
chloramphenicol (CHL)
cinoxacin (CIN)
ciprofloxacin (CIP)
clarithromycin (CLR)
clinafloxacin (CLX)
clindamycin (CLI)
colistin (CST)
dalbavancin (DAL)
daptomycin (DAP)
dicloxacillin (DCX)
dirithromycin (DTM)
doripenem (DOR)
doxycycline (DOX)
enoxacin (ENX)
erythromycin (ERY)
fleroxacin (FLE)
fosfomycin (FOF)
gatifloxacin (GAT)
gentamicin (GEN)
grepafloxacin (GRX)
imipenem (IPM)
kanamycin (KAN)
levofloxacin (LVX)
linezolid (LZD)
lomefloxacin (LOM)
loracarbef (LOR)
meropenem (MEM)
methicillin (MET)
mezlocillin (MEZ)
minocycline (MIN)
moxalactam (MOX)
moxifloxacin (MXF)
nafcillin (NAF)
nalidixic acid (NAL)
netilmicin (NET)
nitrofurantoin (NIT)
norfloxacin (NOR)
ofloxacin (OFX)
oritavancin (ORI)
oxacillin (OXA)
penicillin (PEN)
piperacillin (PIP)
piperacillin-tazobactam (TZP)
polymyxin B (PMB)
quinupristin-dalfopristin (Synercid) (Q-D)
rifabutin (RFB)
rifampin (RIF)
rifapentine (RFP)
sparfloxacin (SPX)
spectinomycin (SPT)
streptomycin (STR)
tedizolid (TZD)
teicoplanin (TEC)
telavancin (TLV)
telithromycin (TEL)
tetracycline (TET)
ticarcillin (TIC)
ticarcillin-clavulanic acid (TIM)
tigecycline (TGC)
tobramycin (TOB)
trimethoprim (TMP)
trimethoprim-sulfamethoxazole (SXT)
trovafloxacin (TVA)
vancomycin (VAN)

(ii) β-Lactamase inhibitors. Use the indicated abbreviations for the following β-lactamase inhibitors.

avibactam (AVI)
clavulanic acid (CLA)
sulbactam (SUL)
tazobactam (TZB)

(iii) Antifungal agents. Use the indicated abbreviations for the following antifungal agents.

amphotericin B (AMB)
anidulafungin (AFG)
caspofungin (CAS)
clotrimazole (CLT)
flucytosine (5FC)
fluconazole (FLC)
isavuconazole (ISA)
itraconazole (ITC)
ketoconazole (KTC)
micafungin (MFG)
nystatin (NYT)
posaconazole (POS)
terbinafine (TRB)
voriconazole (VRC)

(iv) Antiviral agents. Use the indicated abbreviations for the following antiviral agents (for additional agents, refer to Table 2 in De Clercq and Li, Clin Microbiol Rev 29:695–747, 2016 [https://doi.org/10.1128/CMR.00102-15]).

acyclovir (ACV)
cidofovir (CDV)
famciclovir (FCV)
foscarnet (FOS)
ganciclovir (GCV)
penciclovir (PCV)
valacyclovir (VCV)
zidovudine (AZT)

(v) Antimycobacterial agents. Use the indicated abbreviations for the following antimycobacterial agents.

bedaquiline (BDQ)
capreomycin (CAP)
clofazimine (CLO)
D-cycloserine (DCS)
delamanid (DMD)
ethambutol (EMB)
ethionamide (ETO)
isoniazid (INH)
para-aminosalicylic acid (PAS)
prothionamide (PTO)
pyrazinamide (PZA)

The use of "nonstandard" abbreviations to designate names of antibiotics and other pharmaceutical agents generally will not be accepted, because the use of different abbreviations for a single agent has often caused confusion. If, on occasion, a nonstandardized abbreviation for a drug or pharmaceutical substance is used, it will be accepted under the following conditions: (i) it must be defined at the first use in the text, (ii) it must be unambiguous in meaning, and (iii) it must contribute to ease of assimilation by readers.

Chemical or generic names of drugs should be used; the use of trade names is not permitted. Avoid the ambiguous term "generation" when classes of drugs are described. When code names or corporate proprietary numbers are to be used, either the chemical structure of the compound or a published literature reference illustrating the chemical structure, if known, must be provided at the first occurrence of the code name or number. For compounds not identified by generic nomenclature, all previous or concurrent identification numbers or appellations should be listed in the manuscript.

Pharmacodynamic terminology. Pharmacodynamic indices (PDIs) must be introduced at their first occurrence in the text and follow guidelines set forth by Mouton et al. (J Antimicrob Chemother 55:601–607, 2005). In Materials and Methods, it should be clearly stated how the PDIs were derived. The most common indices used are the following: AUC/MIC ratio (the area under the concentration-time curve over 24 h in steady state divided by the MIC), AUIC (the area under the inhibitory curve; note that the AUC/MIC ratio is not

equal to the AUIC), $\%T_{MIC}$ (the cumulative percentage of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions), C_{max}/MIC ratio (the peak level divided by the MIC), PTA (probability of target attainment), and CFR (cumulative fraction of response). Clear distinction should be made between $\%T_{MIC}$, which is expressed as a percentage of the dosing interval, and T_{MIC}, expressed in hours. It is strongly recommended that the prefix *f* be used with an index (e.g., *f*AUC) if the free, unbound fraction of the drug is meant.

β-Lactamases

Studies performed to characterize a β-lactamase or the interaction of a compound with a β-lactamase (i.e., as a substrate, inhibitor, or inducer) should follow the guidelines set forth by Bush and Sykes (Antimicrob Agents Chemother 30:6–10, 1986). Assays that measure the hydrolysis of β-lactam antibiotics must be appropriate for the substrate examined (e.g., iodometric methods are not appropriate quantitative assays for substrates whose products are unknown). Reproducibility of results must be shown. When referring to β-lactamases, please use the functional designations defined by Bush and Jacoby (Antimicrob Agents Chemother 54:969–976, 2010). Alternatively, if the amino acid sequence for the enzyme is known, the β-lactamases may be described by molecular class as initiated by Ambler (Philos Trans R Soc Lond B Biol Sci 289:321–331, 1980).

A database of defining amino acid alterations for many β-lactamases is maintained at https://www.ncbi.nlm.nih.gov/projects/pathogens/submit_beta_lactamase/. The managers of that site should be consulted about the name of a potentially novel β-lactamase sequence before a new designation or number is proposed for publication.

In Vitro Susceptibility Tests

Tabulate results of determinations of minimal inhibitory and bactericidal concentrations according to the range of concentrations of each antimicrobial agent required to inhibit or kill the members of a species or of each group of microorganisms tested, as well as the corresponding concentrations required to inhibit 50 and 90% of the strains (MIC_{50} and MIC_{90}, respectively) and those required to kill 50 and 90% of the strains (MBC_{50} and MBC_{90}, respectively). Geometric mean MICs may also be reported when relevant. The MIC_{50} and MIC_{90} reported should be the actual concentrations tested that inhibited 50 and 90%, respectively, of the strains. They should not be values calculated from the actual data obtained. When only six to nine isolates of a species are tested, tabulate only the MIC range of each antimicrobial agent tested.

If more than a single drug is studied, insert a column labeled "Test agent" between the columns listing the organisms and the columns containing the numerical data and record data for each agent in the same isolate order. Cumulative displays of MICs or MBCs in tables or figures are acceptable only under unusual circumstances.

The percentage of strains susceptible and/or resistant to an antibiotic at its breakpoint concentration may be given only if an appropriate breakpoint has been approved, as by the Clinical and Laboratory Standards Institute (https://clsi.org/). In the absence of approved breakpoints, authors cannot assign breakpoints or use breakpoints from related antibiotics. An exploratory analysis tabulating the percentage of strains inhibited over a range of concentrations is acceptable.

Bactericidal tests must be performed with a sufficient inoculum ($>5 \times 10^5$ CFU/ml) and subculture volume (0.01 ml) to ensure accurate determination of the 99.9% killing endpoint, as described by Pearson et al. (Antimicrob Agents Chemother 18:699–708, 1980) and Taylor et al. (Antimicrob Agents Chemother 23:142–150, 1983). Inoculum size and subculture volume are also critical to studies of combinations of antimicrobial agents.

Synergy is defined in two-dimensional or checkerboard tests when the fractional inhibitory concentration (FIC) or fractional bactericidal concentration (FBC) index (Σ) is $\leq$0.5. In killing curves, synergy is defined as a $\geq$2-$\log_{10}$ decrease in CFU per milliliter between the combination and its most active constituent after 24 h, and the number of surviving organisms in the presence of the combination must be $\geq$2 $\log_{10}$ CFU/ml below the starting inoculum. At least one of the drugs must be present in a concentration which does not affect the growth curve of the test organism when used alone. Antagonism is defined by a ΣFIC or ΣFBC of $>$4.0.

When standard twofold-dilution schemes are used to determine checkerboard interactions, the inherent variability of the method casts doubt on the significance of interactions represented by ΣFICs or ΣFBCs of $>$0.5 but $\leq$4. Therefore, such interactions, if labeled at all, should be termed "indifferent." Alternatively, indices in this range may be described as "nonsynergistic" or "nonantagonistic," as appropriate. The technically imprecise term "additive" should be avoided, as it is too easily misunderstood. See reports by W. R. Greco et al. (Pharmacol Rev 47:331–385, 1995), F. C. Odds (J Antimicrob Chemother 52:1, 2003), and M. D. Johnson et al. (Antimicrob Agents Chemother 48:693–715, 2004) for further discussion of these issues.

For killing curve tests, the minimal, accurately countable number of CFU per milliliter must be stated and the method used for determining this number must be described. In the absence of any drug and with a sample size of 1 ml, this number is 30 (1.5 in $\log_{10}$) CFU. If procedures for drug inactivation or removal have not been performed, the author must state how drug carryover effects were eliminated or quantified. For drugs showing an inoculum effect, mere dilution below the MIC obtained in standard tests is not sufficient.

Clinical Trials

(i) Registration. AAC requires the prospective registration (i.e., before the first patient is enrolled) of a clinical trial in a public trials registry in accordance with guidelines established by the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html). The ICMJE defines a clinical trial as "any research project that prospectively assigns people or a group of people to an intervention, with or without concurrent comparison or control groups, to study the cause-and-effect relationship between a health-related intervention *and* a health outcome."

AAC does not require registration in a particular registry, but the registry chosen must meet the following criteria, in agreement with ICMJE recommendations. It must be (a) accessible to the public free of charge, (b) open to all registrants, (c) managed by a not-for-profit organization, (d) monitored by a mechanism to ensure validity of registration data, and (e) searchable electronically. A registration with missing fields or uninformative terminology will be deemed inadequate.

The registry and the trial registration number must be included at the end of the abstract. If a registration number is available, the authors should state this number the first time a trial acronym is used to refer to the trial being reported or to other trials mentioned in the manuscript.

(ii) Criteria for enrollment. The methods used to find and enroll patients and the criteria for enrollment in a clinical trial should be stated. In addition, the time period (month/year to month/year) of the enrollment should be specified. It should be indicated, if appropriate, that written informed consent was obtained and that the trial was approved by the pertinent committee on human subjects.

(iii) Method of randomization. Randomized, double-blind studies are preferred. Comparisons using historical controls are usually regarded as questionable unless the differences in outcome between the groups are dramatic and almost certainly the result of the new intervention. The rationale for the choice of the control group should be explained. The sample size should be justified, and the method of randomization should be stated.

(iv) Criteria for determining whether a case is evaluable. The minimum criteria for evaluability should be stated explicitly. For example, it should be stated that the minimum criterion for evaluability was *a* or the combination of *b* and *c* rather than *a*, *b*, and *c* without designating which were the minimum criteria. The criteria for evaluability are usually different from those for enrollment.

(v) Reasons for nonevaluability. State the number of patients in each group who were excluded from evaluation and the reason(s) for each exclusion.

(vi) Criteria for assessment. Define each outcome for each category of assessment (e.g., "clinical outcomes were classified as cure, improvement, and failure; microbiological outcomes were classified as eradication, persistence, and relapse"). The frequency and timing of such assessments in relation to treatment should be stated. Specify any changes made in the study regimen(s) during the trial; the results for regimens with and without such modification generally should be stated separately. The criteria (questionnaires, results of specific laboratory tests) for evaluation of adverse effects should be stated, as should the period encompassed in the assessment and the time of assessment in relation to the time of treatment (e.g., daily during treatment). Some authors prefer to consider superinfections as failures of treatment, whereas others prefer to consider them separately or even as adverse effects. In any event, the manuscript should state the number of superinfections with each regimen and should differentiate between superinfections and colonization. The duration of follow-up should be mentioned.

(vii) Statistical analyses. The type of statistical test should be stated, and when appropriate, the reason for the choice of test should be given. References should be given for statistical procedures other than the *t* test, chi-square test, and Wilcoxon rank sum test. The comparability of the treatment groups at the baseline should be evaluated statistically.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the articles by Olsen (Infect Immun 71:6689–6692, 2003; Infect Immun 82:916–920, 2014).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J Virol 79:669–676, 2005).

(viii) Beta error. For trials which show no statistically significant difference between regimens, calculate the probability (β) of a type II error and the power of the study ($1 - \beta$) to detect a specified clinically meaningful difference in efficacy between the regimens. For further details, see the article by Freiman et al. (N Engl J Med 299:690–694, 1978). Alternatively, or in addition, indicate the magnitude of difference between the regimens that could have been detected at a statistically significant level with the number of evaluable patients studied.

For further details, see the editorial on guidelines for clinical trials (Antimicrob Agents Chemother 33:1829–1830, 1989).

Reporting Numerical Data

Standard metric units are used for reporting length, weight, and volume. For these units and for molarity, use the prefixes m, μ, n, and p for 10^{-3}, 10^{-6}, 10^{-9}, and 10^{-12}, respectively. Likewise, use the prefix k for 10^3. Avoid compound prefixes such as mμ or $\mu\mu$. Use μg/ml or μg/g in place of the ambiguous ppm. Units of temperature are presented as follows: 37°C or 324 K.

When fractions are used to express units such as enzymatic activities, it is preferable to use whole units, such as g or min, in the denominator instead of fractional or multiple units, such as μg or 10 min. For example, "pmol/min" is preferable to "nmol/10 min," and "μmol/g" is preferable to "nmol/μg." It is also preferable that an unambiguous form, such as exponential notation, be used; for example, "μmol g^{-1} min^{-1}" is preferable to "μmol/g/min." Always report numerical data in the appropriate SI units.

Representation of data as accurate to more than two significant figures must be justified by presentation of appropriate statistical analyses.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the articles by Olsen (Infect Immun 71:6689–6692, 2003; Infect Immun 82:916–920, 2014).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J Virol 79:669–676, 2005).

Isotopically Labeled Compounds

For simple molecules, labeling is indicated in the chemical formula (e.g., $^{14}CO_2$, $^{3}H_2O$, and $H_2{}^{35}SO_4$). Brackets are not used when the isotopic symbol is attached to the name of a compound that in its natural state does not contain the element (e.g., ^{32}S-ATP) or to a word that is not a specific chemical name (e.g., ^{131}I-labeled protein, ^{14}C-amino acids, and ^{3}H-ligands).

For specific chemicals, the symbol for the isotope introduced is placed in square brackets directly preceding the part of the name that describes the labeled entity. Note that configuration symbols and modifiers precede the isotopic symbol. The following examples illustrate correct usage:

[^{14}C]urea	[γ-^{32}P]ATP
L-[*methyl*-^{14}C]methionine	UDP-[U-^{14}C]glucose
[2,3-^{3}H]serine	*E. coli* [^{32}P]DNA
[α-^{14}C]lysine	fructose 1,6-[1-^{32}P]bisphosphate